Determination of the Level of Aflatoxins Contamination in Maize (Zea mays L.) Produced in Five Regions of Côte d’Ivoire

Sira Bamba1*, Henri Marius Godi Biego1,2, Adama Coulibaly3, Nyamien B. Yves4 and Sidibe Daouda1

1Laboratory Biotechnology, Agriculture and Development of Biological Resources, Training and Research Unit of Biosciences, Felix Houphouet-Boigny University, Abidjan, 22 BP 582 Abidjan 22, Cote d’Ivoire.
2Research Unit of Biochemistry and Food Sciences, Training and Research Unit of Biosciences, Felix Houphouet-Boigny University of Abidjan, 22 BP 582 Abidjan 22, Cote d’Ivoire.
3Department of Public Health, Hydrology and Toxicology, Training and Research Unit of Pharmacological and Biological Sciences, Felix Houphouet-Boigny University, BP 34 Abidjan, Côte d’Ivoire.
4Training and Research Unit of Institute Agropastoral Management, Peleforo Gon Coulibaly University, P.O.Box 1328, Korhogo, Côte d’Ivoire.

Authors’ contributions

This work was carried out in collaboration among all authors. Author SB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AC and SD managed the analyses of the study. Author HMGB managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims: The aim of this work is to assess the level of aflatoxins contamination in maize produced in five regions (Poro, Hambol, Gontougo, Gbéké, Indénié-Djuablin) of Côte d’Ivoire.

Place and duration of study: In this study 375 samples of maize (grains, cobs, spathes) were taken from February 2016 to January 2017 and the aflatoxin analyses were carried out at the
Biotechnology Laboratory, Agriculture and Development of Biological Resources of the Félix Houphouët-Boigny University.

**Methodology:** The aflatoxins (B1, B2, G1 and G2) were extracted and assayed by High Performance Liquid Chromatography (HPLC) according to the AOAC method.

**Results:** The results indicate the presence of aflatoxins B1, B2, G1 and G2 in all forms of maize (grains, cobs, spathes) and in the five regions chosen for the study. The mean concentrations of aflatoxins B1 (AFB1) and the total aflatoxins (AFT) varied from 0.79 ± 0.04 µg/kg to 130.31 ± 22.56 µg/kg and from 2.63 ± 2.35 µg/kg to 169.13 ± 40.39 µg/kg respectively. Samples from Indénié-Djuablin, Hambol and Gountougo regions showed the highest proportions of non-compliance with the European Union limit of 5 µg/kg and 10 µg/kg. Regarding AFB1, these proportions vary from 0% to 46% in the regions of Gbêkê, Poro and Hambol, while they are between 54% and 96% in the regions of Indénié-Djuablin and Gountougo. For total aflatoxins, the proportions of non-compliant samples were between 0% and 40% (Gbêkê and Poro), 12% and 56% (Hambol), 56% and 96% (Indénié-Djuablin and Gountougo).

**Conclusion:** These results demonstrate a need for monitoring of maize production by stakeholders in the sector who should adopt good agricultural practices.

**Keywords:** Maize; grains; cobs; spathes; aflatoxins; sanitary quality; Côte d'Ivoire.

# 1. INTRODUCTION

Access to sufficient, safe, and nutritious food is essential for sustaining life and promoting good health [1]. In fact, food is a source of life as a provider of nutrients essential for the development and well-being of an individual. However, they are also feared and stigmatized as vectors of chemical and/or biological contaminants that can be sources of disease [2,3]. Among these contaminants, some are less well known to the public, in particular the mycotoxins which are produced naturally in food by molds. These natural contaminants have health effects in both humans and animals [4]. Mycotoxins are among the most significant food contaminants in terms of negative impact on public health, food security and the economy of many countries [5,6]. Groups of mycotoxins considered important from an agri-food and health perspective are ochratoxin A, fumonisins, zearalenone, trichothecenes, patulin, deoxynivalenol, toxin T-2 and aflatoxins [7].

Aflatoxins contaminate various categories of foods, of which grains represent the greatest risk factor. This situation is the consequence of their high consumption and the frequency of their contamination [8]. One of the most vulnerable cereals to aflatoxins contamination is maize [9-12]. Aflatoxins have been found in maize produced in several African countries, notably Nigeria (1.5-257.82 µg/kg), Senegal (1.06-852.2 ng/kg), Uganda (86- 3300 ng/kg) and Tanzania (8-1081 ng/kg) [12-14].

In Côte d'Ivoire, studies have shown that the level of aflatoxin contamination in maize during storage varies depending on the nature of the storage material and the quality of the treatments [15,16]. Thus, a better approach in protecting the health of populations would be an assessment of their level of exposure to aflatoxins through the consumption of maize produced and marketed in production areas. So, this study was initiated to determine the level of aflatoxins contamination in maize produced and marketed in Côte d'Ivoire to guarantee food security.

# 2. MATERIALS AND METHODS

## 2.1 Material

### 2.1.1 Biological material

The biological material consists of grains, cobs, and spathes of dried maize from the North, East, Center, Center-North and North-East regions of Côte d'Ivoire.

### 2.1.2 Study sites

The samples were taken in the localities of Gbêkê (Center), Poro (North), Hambol (center-North), Indénié-Djuablin (East) and Gountougo (North-East). The specificities of these regions were given by Bamba et al. [17].

## 2.2 Methods

### 2.2.1 Sampling

The strategy adopted 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 (spathes, cobs and grains) were taken from the stocks of the producers constituting the second phase. The collection of samples was done 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 samples were sent to the laboratory to determine contamination level in aflatoxins.

2.2.2 Determination of the water activity of maize

Water activity of maize was determined as reported by Bamba et al. [18].

2.2.3 Extraction and quantification of aflatoxins from maize

Aflatoxins were solvent extracted and assayed using a high-performance liquid chromatograph (HPLC), fitted with a fluorescence detector, according to the AOAC method [19].

2.2.3.1 Extraction and purification of aflatoxins

In a 250 mL Erlenmeyer flask containing 25 g of maize ground, 100 ml of methanol-water (v/v, 80:20) were added. The mixture was homogenized by shaking for 2 minutes and then stored at room temperature in the dark for 12 hours. The homogenate was filtered through filter paper and 50 ml of the filtrate were added 40 ml of phosphotungstic acid-zinc sulfate-water mixture (w/w/v; 5/15/980) then stored at room temperature for 15 minutes. The mixture was filtered through filter paper and aflatoxins were extracted from the filtrate with 3 volumes of 10 ml of chloroform. The extracts were collected and evaporated to dryness using a rotary evaporator (Buchi, Rotavapor R-215) at 40°C. A dry extract was added 0.4 mL of hydrochloric acid and 4.6 mL of distilled water. The mixture was filtered through a Rezist filter and purified through an immunoaffinity column (RIDA aflatoxin, Biopharm, Germany) containing an antibody specific for aflatoxins (AFB1, AFB2, AFG1 and AFG2) at a flow rate of 2 mL.min⁻¹.

2.2.3.2 Quantification of aflatoxin concentrations

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

| Regions               | Grains | Cobs | Spathes |
|-----------------------|--------|------|---------|
| Gbéké                 | 25     | 25   | 25      |
| Poro                  | 25     | 25   | 25      |
| Hambol                | 25     | 25   | 25      |
| Indénié-Djuablin      | 25     | 25   | 25      |
| Gontougo              | 25     | 25   | 25      |
| Total                 | 125    | 125  | 125     |

Table 1. Number of samples taken depending on the form of maize and the regions of production

| ITEM                      | Aflatoxins (B1, B2, G1 and G2)                                                                 |
|---------------------------|-----------------------------------------------------------------------------------------------|
| 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                                         |
| Mobile phase              | Acetonitrile/Water/Methanol (20/20/60)                                                          |
| Injection volume          | 20 μL                                                                                            |
| Flow rate                 | 1 mL/min at isocratic                                                                        |
| Temperature of the column | 40 °C                                                                                            |
| Rinsing solvent           | Methanol                                                                                        |
| Duration of analysis      | 15 minutes                                                                                     |
2.2.3.3 Validation of the HPLC method of aflatoxin analysis

The HPLC method validation was conducted following the method of the French Association for Standardization [20]. 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 (0.10 ng/kg, 4.5 ng/kg, 10 ng/kg et 20 ng/kg). The reference material (aflatoxin standards) was used to compare the concentration of aflatoxins obtained at the certified value.

2.3 Statistical Analysis

The tests were carried out in triplicate and the averages were calculated. The homogeneity of the means was assessed from the Student-Newman-Keuls test at 5% risk using SPSS software version 20.0. The percentages of non-compliant samples made it possible to assess the occurrence of AFB1 and AFT in the different regions. Finally, the correlation between the aflatoxins and the samples was established by principal component analysis (PCA), using the STATISTICA 7.1 software.

3. RESULTS

3.1 Maize Water Activity

The mean values of the water activity are given 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 were 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 Gbéké and Poro Regions show lowered water activity while those from Indénié-Djuablin and Gontougo had high values.

### Table 3. Average values of water activity according to the maize form and production regions

| Regions           | Grains     | Cobs      | Spathes   | F_value | P_value |
|-------------------|------------|-----------|-----------|---------|---------|
| Gbéké             | 0.80±0.02  | 0.81±0.02 | 0.84±0.05 | 5.97    | <0.001  |
| Poro              | 0.78±0.01  | 0.81±0.03 | 0.85±0.05 | 19.53   | <0.001  |
| Hambol            | 0.81±0.01  | 0.84±0.02 | 0.90±0.06 | 29.29   | <0.001  |
| Indénié-Djuablin  | 0.93±0.03  | 0.88±0.06 | 0.93±0.05 | 9.91    | <0.001  |
| Gontougo          | 0.92±0.05  | 0.85±0.05 | 0.89±0.04 | 15.84   | <0.001  |
| F_value           | 95.84      | 95.84     | 13.22     | nd      | nd      |
| P_value           | <0.001     | <0.001    | <0.001    | nd      | nd      |

Means with the same letters are statistically identical. Lowercase and uppercase letters are representative of columns and rows, respectively.

3.2 Validation of the Aflatoxin Determination Method

The Pearson coefficients (R^2) determined for linearity are between 0.98 and 0.99. The detection limits are 6.18 ng/kg, 0.058 ng/kg, 114.5 ng/kg and 2.64 ng/kg respectively for aflatoxins B1, B2, G1 and G2. As for the quantification limits, they are respectively 6.50 ng/kg, 0.108 ng/kg, 124.9 ng/kg and 2.94 ng/kg for aflatoxins B1, B2, G1 and G2. The coefficients of variation calculated for the repeatability tests vary between 0.50 ± 0.00% and 3.75 ± 0.22% while those calculated for the reproducibility tests are between 0.89 ± 0.10% and 4.93 ± 0.37%. The extraction yields obtained are 98.92 ± 2.49%; 97.53 ± 1.93%; 95.31 ± 0.33% and 97.63 ± 2.09% respectively for aflatoxins B1, B2, G1 and G2 (Table 4).

3.3 Concentrations of Aflatoxins in the Samples

Table 5 shows the concentrations of the various aflatoxins found in the maize grains, cobs and spathes samples. Aflatoxin concentrations varied regardless of region and form of maize. Concerning aflatoxin B1, the levels are between 0.79 ± 0.04 µg/kg and 20.92 ± 4.63 µg/kg; 2.26 ± 1.39 µg/kg and 32.22 ± 10.40 µg/kg; 12.73 ± 6.25 µg/kg and 130.31 ± 22.56 µg/kg respectively for grains, cobs and spathes. Average concentrations of less than 5 µg/kg are obtained in the regions of Gbéké, Poro and Hambol for maize grains and cobs, while those obtained in the regions of Indénié-Djuablin and Gountougo are above this value whatever the spathes of maize.
As for aflatoxins B2, G1, and G2, their levels determined on the grains, cobs and spathes of maize, collected in the regions of Gbêkê, Poro and Hambol, were relatively low. The concentrations of aflatoxin B2 and aflatoxin G2 are all less than 5 µg/kg, and they vary between 0.08 ± 0.04 µg/kg and 3.40 ± 1.84 µg/kg regardless of the region. For the contents of aflatoxin G1, the values for the grains, cobs and spathes, vary respectively from 1.64 ± 0.57 µg/kg to 37.10 ± 18.84 µg/kg; 3.75 ± 1.85 µg/kg at 30.31 ± 15.58 µg/kg and from 3.85 ± 0.95 µg/kg at 32.31 ± 17.58 µg/kg.

Regarding total aflatoxins, the concentrations obtained vary from 2.63 ± 2.35 µg/kg to 169.13 ± 40.39 µg/kg. Concentrations of less than 10 µg/kg are obtained on maize grains (Gbêkê, Poro and Hambol) and the cobs (Gbêkê and Poro).

### Table 4. Validation of the aflatoxin determination method

| Designation  | AFB1 (µg/kg) | AFB2 (µg/kg) | AFG1 (µg/kg) | AFG2 (µg/kg) |
|--------------|--------------|--------------|--------------|--------------|
| Linearity (R²) | 0.99         | 0.98         | 0.98         | 0.99         |
| Limit of detection (ng/kg) | 6.18          | 0.058        | 114.5        | 2.64         |
| Limit of quantification (ng/kg) | 6.50          | 0.108        | 124.9        | 2.94         |
| Repeatability (CV, %) | 2.08±0.10    | 3.75±0.22    | 0.50±0.00    | 3.56±0.18    |
| Reproducibility (CV, %) | 3.20±0.18    | 4.93±0.37    | 0.89±0.10    | 4.72±0.15    |
| Extraction yield (EY, %) | 98.92±2.49   | 97.53±1.93   | 95.31±0.33   | 97.63±2.09   |

### Table 5. Aflatoxin concentrations according to the different forms of maize and regions (µg/kg)

#### Aflatoxin B1 concentration (AFB1)

| Regions       | Grains | Cobs | Spathes | F-value | P-value |
|---------------|--------|------|---------|---------|---------|
| Gbêkê         | 1.97±0.69<sup>aA</sup> | 2.26±1.39<sup>aA</sup> | 12.73±6.25<sup>ad</sup> | 4.01 | 0.001 |
| Poro          | 0.79±0.04<sup>aA</sup> | 2.52±0.93<sup>aA</sup> | 18.27±5.96<sup>ad</sup> | 7.18 | 0.001 |
| Hambol        | 1.31±0.36<sup>aA</sup> | 4.73±1.33<sup>aA</sup> | 19.91±8.48<sup>ad</sup> | 15.91 | 0.001 |
| Indéné-Djuablin | 9.30±2.76<sup>da</sup> | 32.22±10.40<sup>db</sup> | 130.31±22.56<sup>ac</sup> | 27.66 | 0.001 |
| Gontougo      | 20.92±4.63<sup>cA</sup> | 13.77±4.45<sup>cA</sup> | 55.41±14.10<sup>cd</sup> | 0.46 | 0.001 |
| F-value       | 10.62   | 6.20 | 19.64   | nd      | nd      |
| P-value       | 0.001   | 0.001| 0.001   | nd      | nd      |

#### Aflatoxin B2 concentration (AFB2)

| Regions       | Grains | Cobs | Spathes | F-value | P-value |
|---------------|--------|------|---------|---------|---------|
| Gbêkê         | 0.18±0.08<sup>aA</sup> | 0.38±0.06<sup>aA</sup> | 0.52±0.16<sup>aA</sup> | 2.84 | 0.064 |
| Poro          | 0.10±0.01<sup>aA</sup> | 0.28±0.027<sup>acd</sup> | 0.40±0.03<sup>ad</sup> | 4.87 | 0.010 |
| Hambol        | 0.19±0.02<sup>aA</sup> | 0.56±0.05<sup>aA</sup> | 0.53±0.20<sup>aA</sup> | 1.39 | 0.250 |
| Indéné-Djuablin | 0.58±0.07<sup>aA</sup> | 3.20±1.83<sup>ab</sup> | 3.39±1.04<sup>bA</sup> | 3.62 | 0.036 |
| Gontougo      | 1.24±0.4<sup>aA</sup> | 1.50±0.60<sup>aA</sup> | 1.70±0.68<sup>bA</sup> | 4.01 | 0.250 |
| F-value       | 7.77    | 5.98 | 5.98    | nd      | nd      |
| P-value       | 0.001   | 0.001| 0.001   | nd      | nd      |

#### Aflatoxin G1 concentration (AFG1)

| Regions       | Grains | Cobs | Spathes | F-value | P-value |
|---------------|--------|------|---------|---------|---------|
| Gbêkê         | 3.35±1.52<sup>aA</sup> | 3.75±1.85<sup>aA</sup> | 3.85±0.95<sup>aA</sup> | 0.11 | 0.88  |
| Poro          | 1.64±0.57<sup>aA</sup> | 4.07±2.51<sup>ab</sup> | 4.17±2.51<sup>ab</sup> | 3.42 | <0.001 |
| Hambol        | 2.34±1.33<sup>aA</sup> | 8.70±3.30<sup>aA</sup> | 10.80±3.15<sup>ab</sup> | 1.36 | <0.001 |
| Indéné-Djuablin | 16.06±7.45<sup>cA</sup> | 30.31±15.58<sup>cA</sup> | 32.31±17.58<sup>cA</sup> | 2.36 | <0.001 |
| Gontougo      | 37.10±18.84<sup>cA</sup> | 26.56±10.44<sup>cA</sup> | 27.56±11.44<sup>cA</sup> | 5.60 | <0.001 |
| F-value       | 10.59   | 4.35 | 4.35    | nd      | nd      |
| P-value       | 0.001   | 0.001| 0.001   | nd      | nd      |

#### Aflatoxin G2 concentration (AFG2)

| Regions       | Grains | Cobs | Spathes | F-value | P-value |
|---------------|--------|------|---------|---------|---------|
| Gbêkê         | 0.17±0.05<sup>aA</sup> | 0.53±0.07<sup>ab</sup> | 0.48±0.06<sup>ab</sup> | 4.49 | 0.014 |
| Poro          | 0.08±0.04<sup>aA</sup> | 0.50±0.01<sup>ab</sup> | 0.38±0.07<sup>ab</sup> | 21.06 | 0.001 |
| Hambol        | 0.16±0.10<sup>aA</sup> | 0.63±0.04<sup>aA</sup> | 0.66±0.20<sup>aA</sup> | 1.87 | 0.160 |
| Indéné-Djuablin | 0.50±0.44<sup>aA</sup> | 3.29±1.04<sup>ab</sup> | 3.40±1.84<sup>ab</sup> | 3.79 | 0.026 |
3.4 Proportion of Non-compliant Samples with European Standards on Aflatoxins

The reference standards defined relate only to aflatoxins B1 and total with limit values of 5 µg/kg and 10 µg/kg respectively. The samples from the Indénié-Djuablin, Hambol and Gontougo regions show the highest proportions of non-compliance. Regarding AFB1, these proportions vary from 0% to 46% in the regions of Gbêkê, Poro and Hambol whatever the form of the maize, while they are between 54% and 96% in the regions of Indénié-Djuablin and Gontougo. For total aflatoxins, the proportions of non-compliant samples are between 0% and 40% (Gbêkê and Poro), 12% and 56% (Hambol), 56% and 96% (Indénié-Djuablin and Gontougo) whatever the form of maize (Table 6).

Table 6. Proportion of non-compliant samples according to the forms of maize and the regions (Percentage)

| Regions          | Aflatoxin B1 | Total aflatoxins |
|------------------|--------------|------------------|
|                  | Grains       | Cobs             | Spathes |
| Gbêkê            | 20           | 24               | 20      |
| Poro             | 0            | 20               | 36      |
| Hambol           | 4            | 20               | 46      |
| Indénié-Djuablin| 60           | 68               | 96      |
| Gontougo         | 76           | 54               | 76      |
| Norme de référence (µg/kg) | 5 |                   |
|                  |              |                  |
|                  |              |                  |

Means with the same letters are statistically identical. Lowercase and uppercase letters are representative of columns and rows respectively.

3.5 Variability of Quality Parameters of the Maize

Table 7 shows that components F1 and F2 of the principal component analysis express 91.36% of the total variability of quality parameters of the maize forms. Component F1, with an eigenvalue of 4.93, expresses 82.19% of the total variability, while component F2 with eigenvalue 0.55 expresses 9.17%. The projection of the quality parameters in the F1-F2 factorial plane shows strong negative correlations between the concentrations of AFB1, AFB2, AFG1, AFG2, AFT and water activity of maize with component F1 (Fig. 1A). While the projection of the maize samples, in the same plane, indicates a split into two groups (Fig. 1B). Group 1 is made up of 5 individuals with high levels of aflatoxins and water activity.
Fig. 1. Projection of quality parameters (A) and the different forms of maize (B) in the factorial plane 1-2 of the principal component analysis

GKé : Gbéké ; PRo : Poro ; HBo : Hambol ; IDj : Indéné-Djuablin ; GTg : Gontougo ; G : Grains ; E : Cobs ; S: Spathes ; AFB1 : Aflatoxin B1 ; AFB2 : Aflatoxin B2 ; AFG1 : Aflatoxin G1 ; AFG2 : Aflatoxin G2 ; AFT : Total Aflatoxins, Aw : Water activity
respectively from 0.058 ng/kg to 114.5 ng/kg and limits of detection and quantification vary analyte and the response of the device. The good correlation between the amount of the determined are great samples. All the Pearson’s coefficients (R) for determining aflatoxins in maize method validation demonstrated the reliability of the method for determining aflatoxins in maize (cobs or grains). Thus, to avoid the development of molds, the different forms of the maize (cobs or grains). The contamination of these toxins in the samples can be explained by the fact that the post-harvest conditions of the maize favor the development of toxinogenic molds. The surveys carried out by Niamketchi et al [15] in three production areas reported failures in the post-harvest system, in particular poor drying, attacks by insects, rodents, termites, and molds.

Aflatoxins B1, B2, G1 and G2 have been detected and quantified in maize regardless of the production region and the form of the maize (grains, cobs, spathes). The contamination of maize by aflatoxins has been reported by several authors in Côte d’Ivoire [25-29]. The prevalence of these toxins in the samples can be explained by the fact that the post-harvest conditions of the maize favor the development of toxinogenic molds. The surveys carried out by Niamketchi et al [15] in three production areas reported failures in the post-harvest system, in particular poor drying, attacks by insects, rodents, termites, and molds.

According to these authors, these failures are the result of failure to respect basic principles such as drying maize, depositing it on dunnage and ventilating storage rooms. In addition, they also mentioned the storage, in the same structure, of foodstuffs from various horizons. This practice is said to be a potential source of secondary infestation. However, the contamination levels of aflatoxins B2, G1 and G2 in the samples are relatively low regardless of the region and form of the maize. On the other hand, the aflatoxins B1 and total have higher levels of contamination whatever the zone. Nevertheless, variability is observed between production regions and this in terms of the proportions of samples that do not comply with European standards. The Gbéké and Poro regions contain less than 50% of samples that do not comply with the various standards (AFB1 and AFT) regardless of the form of the maize. While the regions of Hambol, Indéné-Djuablin and Gontougo contain more than 56% regardless of the standard and form of maize. This contamination variability could be explained by the variability in the level of water activity observed in the different regions. This is because the principal component analysis indicates a strong correlation between water activity values vary between 0.78 and 0.93 regardless of the form of the maize and the region of production. This variability could be due to the hygrometry of the regions since they have different geographic specificities [17]. According to Comelade and Yoka et al. [21,22], the production and post-harvest stages of an agricultural product are influenced by environmental conditions (climate, temperature, rainfall). The water activity of agricultural products makes a decisive contribution to accelerating or delaying the phenomena of biochemical transformations that are at the origin of grains deterioration. Because according to the FAO [8], this degradation depends mainly on several factors including humidity, temperature, water activity and oxygen in the environment. In addition, Niamketchi [23] demonstrated strong correlations (0.62-0.90) between the water activity of maize and the nutritional and health parameters (mycotoxins) whatever the form of the maize (cobs or grains). Thus, to avoid the development of molds, the different forms of maize must have a water activity of less than 0.65 [5]. Because toxinogenic molds such as Aspergillus flavus and Aspergillus parasiticus can develop in all the climates, on all the solid or liquid supports as soon as there are nutrients, humidity, and water activity greater than 0.65 [7,24].

Method validation demonstrated the reliability of the method for determining aflatoxins in maize samples. All the Pearson’s coefficients (R²) determined are greater than 0.98, indicating a good correlation between the amount of the analyte and the response of the device. The limits of detection and quantification vary respectively from 0.058 ng/kg to 114.5 ng/kg and from 0.108 ng/kg to 124.9 ng/kg, which indicates good sensitivity of the assay device. Additionally, the coefficients of variation determined for repeatability and reproducibility are all less than 6% and all extraction rates are greater than 90%. This situation indicates that the aflatoxins extraction and assay methods are reliable.

### Table 7. Eigenvalues and variability of the parameters

| Designation      | 1     | 2     | 3     | 4     | 5     | 6     |
|------------------|-------|-------|-------|-------|-------|-------|
| Eigenvectors     | 4.93  | 0.55  | 0.41  | 0.09  | 0.01  | 0.00  |
| Variability (%)  | 82.19 | 9.17  | 6.85  | 1.49  | 0.25  | 0.05  |
| Cumulative variability (%) | 82.19 | 91.36 | 98.21 | 99.71 | 99.95 | 100.00 |

These are maize spathes from the regions of Gontougo and Indéné-Djuablin; maize grains from Gontougo and maize cobs from Indéné-Djuablin and Gontougo. Group 2 contains 10 individuals showing low levels of aflatoxins and water activity including spathes, cobs and grains of maize from the Gbéké, Poro and Hambol regions as well as the grains of Indéné-Djuablin.

4. DISCUSSION

The water activity values vary between 0.78 and 0.93 regardless of the form of the maize and the region of production. This variability could be due to the hygrometry of the regions since they have different geographic specificities [17]. According to Comelade and Yoka et al. [21,22], the production and post-harvest stages of an agricultural product are influenced by environmental conditions (climate, temperature, rainfall). The water activity of agricultural products makes a decisive contribution to accelerating or delaying the phenomena of biochemical transformations that are at the origin of grains deterioration. Because according to the FAO [8], this degradation depends mainly on several factors including humidity, temperature, water activity and oxygen in the environment. In addition, Niamketchi [23] demonstrated strong correlations (0.62-0.90) between the water activity of maize and the nutritional and health parameters (mycotoxins) whatever the form of the maize (cobs or grains). Thus, to avoid the development of molds, the different forms of maize must have a water activity of less than 0.65 [5]. Because toxinogenic molds such as Aspergillus flavus and Aspergillus parasiticus can develop in all the climates, on all the solid or liquid supports as soon as there are nutrients, humidity, and water activity greater than 0.65 [7,24].

Method validation demonstrated the reliability of the method for determining aflatoxins in maize samples. All the Pearson’s coefficients (R²) determined are greater than 0.98, indicating a good correlation between the amount of the analyte and the response of the device. The limits of detection and quantification vary respectively from 0.058 ng/kg to 114.5 ng/kg and from 0.108 ng/kg to 124.9 ng/kg, which indicates good sensitivity of the assay device. Additionally, the coefficients of variation determined for repeatability and reproducibility are all less than 6% and all extraction rates are greater than 90%. This situation indicates that the aflatoxins extraction and assay methods are reliable.
activity and aflatoxins contamination. This relationship was noted by Niamketchi et al. [28] in their study on the storage conditions for maize. This contamination variability could also be explained by the practice of producers in each region. Because the main component analysis indicates a strong aflatoxins contamination of maize from the Gontougo region regardless of the form of the maize (grains, cobs, spathes). This region is followed by that of the Indénéi-Djuablin (cobs, spathes). Niamketchi et al. [15] noted a difference in the post-harvest practices of producers in the survey carried out in three production zones in Côte d'Ivoire. In addition, overall, spathes represent the most contaminated form of maize in terms of concentration and non-compliant samples. This situation could be explained by the double role of spathes, which is to ensure the natural protection of the spathes against insects and to constitute a barrier to the direct action of pesticide treatments and a brake on the adequate drying of the grains [10,30].

These results suggest an important need for monitoring cereals and other foodstuffs (oil seeds and legumes) against toxigenic molds. Maize contamination can be attributed to agricultural practices (handling during harvest) or storage conditions (temperature, humidity). Actors in the maize sector must apply good agricultural and post-harvest practices to obtain maize of good marketability and health. Thus, the search for alternative storage methods, as indicated by Niamkethi [23] and Pierre et al., [16], could be a solution to reducing the level of mycotoxin contamination of merchant maize.

5. CONCLUSION

The determination of the level of aflatoxins contamination in maize produced in Côte d'Ivoire made it possible to demonstrate the presence of these mycotoxins in all the 5 production regions (Gbékê, Poro, Hambol, Indénéi-Djuablin, Gontougo) and in all the 3 forms of maize (grains, cobs, spathes). This contamination depends on the region of production and the regions of Gbékê and Poro contain 0-46% of samples that do not comply with European standards on the AFB1 and the AFT whatever the form of maize. Contamination levels are higher in spathes and cobs from the Indénéi-Djuablin and the Gontougo as well as grains from the Gontougo. These results should give rise to an urgent need for monitoring this cereal against mycotoxins. This could contribute to a subsequent reduction of aflatoxins contents. Because with regard to the carcinogenic, genotoxic effects without threshold of these mycotoxins, the only realistic approach is to reduce exposure to as low a level as possible according to the ALARA principle (As Low As Reasonably Achievable).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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