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Investigation of the occurrence of mycotoxins in staple foods consumed in Kinshasa (DRC) by an LC-MS/MS-based multimycotoxin analytical approach

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ABSTRACT

Introduction

Mycotoxins are harmful contaminants for human and animal health; their presence also impacts crop production and economies worldwide. They are frequently present in various African staple foods.

Purpose

In this study, a multi-mycotoxin analysis approach was implemented to reveal mycotoxin contamination of important staple foods in Kinshasa (Democratic Republic of the Congo).

Methods

A multi-mycotoxin analysis approach based on liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray interface operated in positive mode (ESI+) and quadrupole mass analyzer was used. Twenty-five mycotoxins were investigated in 18 samples of staple foods collected in different markets in Kinshasa city, including maize flour (n=8), cassava flour (n=5), and peanut paste (n=5).

Results

Twenty mycotoxins were present in these samples at quantifiable levels. The maize flour samples were the most contaminated with aflatoxin B1 (AFB1) (87.5%), total aflatoxin (AFT) (100%), fumonisin B1 (FB1) (87.5%), deoxynivalenol (DON) (75%), and nivalenol (NIV) (75%). Emerging mycotoxins such as beauvericin (BEA), enniatin B (ENN B), alternariol (AOH), and alternariol mono-methyl ether (AME) were more frequent in maize flour. The study found that 25% of maize flour and 20% of peanut paste samples exceeded the European Commission's regulatory limits for aflatoxin B1 (AFB1) and total aflatoxins (AFT). Additionally, ochratoxin A (OTA) was detected in one maize sample at a concentration above the regulatory limit. This could expose consumers to their genotoxic, teratogenic, and immunosuppressive effects. As for OTA, there are fears of its harmful effects, including nephrotoxicity and mutagenicity. The aflatoxin/fumonisin (AF/F) combination was more frequently observed (50% of all samples) than AF/DON, DON/F, and AF/OTA combinations.

Conclusion

Data from this exploratory study highlight a possible health risk for the population of Kinshasa through the consumption of maize flour, as well as the need for continuous monitoring of mycotoxins in this staple food that is increasingly being consumed across the Democratic Republic of the Congo.

INTRODUCTION

Mycotoxins are secondary metabolites produced mainly by fungi of the genera *Aspergillus*, *Fusarium*, and *Penicillium* (Crudo et al., 2019). These microorganisms grow especially on cereals and oilseeds under favorable conditions such as warm and humid climates (Agriopoulou et al., 2020). They can thus produce toxins called mycotoxins in the field, during, and after harvest (Agriopoulou et al., 2020). Currently, several hundred mycotoxins of various families have been identified (Mihalcea & Amariei, 2022).

In this exploratory study, some mycotoxins of toxicological relevance were investigated. The first group, the major mycotoxins, has been known for a long time, is more widespread and regulated both by the European Commission (EC) (EC, 2013), the Food and Drug Administration, and some countries throughout the world, and is the subject of routine analysis (Agriopoulou et al., 2020). This group includes aflatoxins (AF) and ochratoxin (OTA) (mycotoxins from *Aspergillus*), fumonisin (F), deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEN) (mycotoxins from *Fusarium*). These mycotoxins can cause several acute and chronic intoxications that are sometimes fatal in humans and animals. Cases of acute aflatoxin intoxications were reported in Kenya and Tanzania in 2004 and 2016 respectively, causing the deaths of more than a hundred people. Each time, contaminated maize was involved (Chilaka et al., 2022).

As for chronic intoxications by the main mycotoxins, there are fears of toxic effects including carcinogenicity, estrogenicity, neurotoxicity, genotoxicity, nephrotoxicity, immunotoxicity, and hepatotoxicity (IARC, 2002; EFSA, 2014; Hamid et al., 2013; Ropejko & Twarużek, 2021; Groopman et al., 2021; Gbashi et al., 2017; Zinedine et al., 2007; Dellafiora et al., 2017). The second group concerns the so-called emerging mycotoxins, recently revealed thanks to increasingly specific and sensitive detection techniques. These mycotoxins are less studied, not regulated at the moment, and less frequently included in routine analysis. Beauvericin (BEA), enniatin B (ENN B) (metabolites of *Fusarium*) as well as alternariol (AOH) and alternariol mono-methyl ether (AME) (metabolites of *Alternaria*) belong to this group (Agriopoulou et al., 2020). As for their toxicity, BEA and ENN B, for instance, can cause cytotoxic and apoptotic effects on human and animal cell lines (Chilaka et al., 2017).

In addition to their toxic effects on human and animal health, mycotoxins cause economic losses. Since the 1980s, the FAO has estimated global contamination of food crops at 25% (Kępińska-Pacelik and Biel, 2021), while Eskola et al. (2020) currently estimate it between 62 and 80%, given the negative impact of climate change and the current use of much more sensitive analytical methods (Nji et al., 2022a). This presence causes economic losses impacting animal and plant production as well as international trade. In the USA, for example, losses from mycotoxins amount to approximately USD 1.4 billion per year (Nji et al., 2022b). Given strict mycotoxin regulations in the EU, exporters of processed food products there spend USD 670 million a year (Gbashi et al., 2018). In Africa, 50% of cereal production contains aflatoxins in concentrations that exceed international standards (Dieme et al., 2016; Meijer et al., 2021). In addition, the African continent incurs costs of more than USD 750 million a year in the fight against this mycotoxin (Gbashi et al., 2018).

Whether major or emerging, all these mycotoxins can contaminate various foods such as maize and cassava, staple foods for which there is an increasing need across the country. It goes without saying that this situation exposes, de facto, the 12 million inhabitants of the city of Kinshasa to the risks of poisoning (DRC, 2018; 2020). In the DRC, the oldest studies on the identification and quantification of mycotoxins date back to the 1970s and focused in particular on cereals, tubers, and legumes (Ilunga et al., 2022). On the other hand, the most recent studies were carried out between 2013 and 2018 and concerned a few cities and districts such as Kinshasa (Kamika et al., 2014), Lubumbashi (Mulunda et al., 2013), Kabare, and Uvira (Udomkun et al., 2018).

The limited amounts of the aforementioned studies have produced fragmentary information, almost all of which focus on aflatoxins without taking into account other mycotoxins, both major and emerging, which are nonetheless present in foodstuffs. In addition, all these studies were based on a single-mycotoxin approach whereas multi-mycotoxin analysis is currently the most suitable approach. The latter allows, in a single cycle, not

only to carry out the analysis of multiple mycotoxins, but also to reveal the possible co-occurrence of major and emerging mycotoxins. Indeed, the concomitant presence of several mycotoxins in the same food can generate antagonistic, additive, or even synergistic effects (Smith et al., 2016; Alassane-Kpembi et al., 2017).

Numerous studies carried out throughout the world have attempted to evaluate the contamination and toxicological effects of mycotoxins individually. This single-mycotoxin approach has also influenced legislation that does not take into account the combined effects of mycotoxins when setting up the regulatory limits. No study carried out in the DRC has been able to highlight the co-occurrence of the different mycotoxins. Hence, we proposed the detection and quantification of the major (regulated) and emerging (unregulated) mycotoxins in samples of maize flour, cassava flour, and peanut paste collected from the markets of Kinshasa using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in order to acquire knowledge on the prevalence and co-occurrence of multiple mycotoxins.

METHODS

Collection of Samples

Samples were collected in Kinshasa during the dry season from four major markets: Central, Gambela, Matete, and Somba Zigida. A total of 18 samples were collected, including maize flour (n=8), cassava flour (n=5), and peanut paste (n=5). They were all kept in dry, cool conditions until analysis to prevent further contamination.

Reagents and Standards

Water was obtained from an ultrapure purification system (Arium pro system, Sartorius, Goettingen, Germany). LC-MS grade methanol and acetonitrile were purchased from Biosolve BV (Valkenswaard, The Netherlands), n-hexane was obtained from Hipersolv Chromanorm, and ammonium acetate was supplied by VWR International (Zaventem, Belgium). Formic acid and glacial acetic acid (100%) were obtained from Merck (Darmstadt, Germany). Certified mycotoxin standard solutions in acetonitrile were obtained from Biopure (Coring System Diagnostix, Gernsheim, Germany), including zearalanone 9.91 µg/mL (internal standard), ochratoxin A 10.05 µg/mL, aflatoxin mix (aflatoxin-B1, B2, G1, and G2) 19.9, 19.9, 20.1, and 20.1 µg/mL respectively, de-epoxy-deoxynivalenol (internal standard), and sterigmatocystin (STE) 50.9 µg/mL, HT2-toxin (HT-2) 100.2 µg/mL, diacetoxyscirpenol (DAS) 100.3 µg/mL, deoxynivalenol 100.4 µg/mL, 15-acetyldeoxynivalenol (15-AcDON) and zearalenone 100.5 µg/mL, fusarenon-X (FUS-X) 100.6 µg/mL, 3-acetyldeoxynivalenol (3-AcDON) and T2-toxin (T-2) 100.7 µg/mL, nivalenol 103.9 µg/mL, and neosolaniol (NEO) 104.7 µg/mL. The fumonisin mix (FB1 and FB2) 50.3 and 49.3 µg/mL was a certified mycotoxin standard solution in acetonitrile/water (50/50, v/v) from Biopure (Coring System Diagnostix, Gernsheim, Germany). Altenuene (ALT), AOH, and AME were obtained from Sigma (Bornem, Belgium), and roquefortine-C (ROQ-C) was purchased from Alexis Biochemicals (Enzo Life Sciences BVBA, Zandhoven, Belgium). FB3 was obtained from Promec unit (Tygerberg, South Africa).

Stock solutions of ALT and ROQ-C (1 mg/mL) were prepared in methanol. AOH and AME stock solutions (1 mg/mL) were prepared in methanol/dimethylformamide (60/40, v/v). The stock solution of FB3 (1 mg/mL) was prepared in acetonitrile/water (50/50, v/v). All stock solutions were stored for 1 year or until the expiration date at -18°C, except for FB3, which was stored at 4°C. Working standard solutions were made by diluting the stock standard solutions in methanol and were stored at -18°C for 3 months. From the individual stock standard solutions and working solutions, a standard mixture was prepared with the following concentrations: DAS (0.5 ng/µL); ROQ-C (1 ng/µL); aflatoxin B1, B2, G1, and G2 (2 ng/µL); 15-AcDON (2.5 ng/µL); OTA, 3-AcDON, ALT, and STE (5 ng/µL); ZEN, T-2, HT-2, NEO, and AOH (10 ng/µL); NIV, FUS-X, and AME (20 ng/µL); FB3 (25 ng/µL); DON, FB1, and FB2 (40 ng/µL).

Sample Preparation

The sample preparation procedure was adapted from Monbaliu et al. (2010). Briefly, 5g of the sample were weighed in a 50 ml extraction tube. Fifty µL of zearalanone (10 µg/mL) and 25 µL of de-epoxy-deoxynivalenol (50 µg/mL), used as internal standards, were added to the samples. The extraction was carried out using 20 ml of acetonitrile/water/acetic acid (79/20/1, v/v/v) and was followed by defatting with 10 ml of hexane. One part of the defatted extract was filtered through a glass filter

while the other was purified on a MultiSep 226 AflaZON+ multifunctional column (Romer Labs, Gernsheim, Germany). Two ml of the filtered defatted extract was combined with the MultiSep 226 eluate. After solvent evaporation at 40 °C under a stream of nitrogen, the residue was dissolved in 150 µL of injection solvent consisting of a 60/40, v/v mixture of mobile phases A and B (see section LC-MS/MS condition). The reconstituted extract was filtered using an Ultrafree PVDF Centrifugal Filter Unit (Millipore Bedford, MA, USA) at 870 x g for 15 min, and the filtrate was transferred to an LC-MS vial.

LC-MS/MS Conditions

A Waters Acquity UPLC system coupled to a Quattro Premier XE triple-quadrupole mass spectrometer (Waters, Milford, MA, USA) was used to analyze the samples. The chromatographic separation was achieved using a 150 mm x 2.1 mm i.d. 5 µm Symmetry C18 column with a 10 mm x 2.1 mm i.d. guard column (Waters, Zellik, Belgium). Two mobile phases, A and B, consisting of water, methanol, and acetic acid in the respective proportions of 94/5/1 (v/v/v) and 2/97/1 (v/v/v) and containing each 5 mM ammonium acetate buffer, were used in gradient elution mode at a flow rate of 0.3 ml/min. The gradient elution program was as follows: 0–7 min, 5–65% B; 7–11 min, 65–75% B; 11–13 min, 75–100% B; 13–14 min, 100% B; 14–16 min, 100–60% B; 16–24 min, 60–40% B; 24–26 min, 40–95% B; 26–28 min, 95% B. The injection volume was 10 µL. Electrospray ionization (ESI) was used in positive mode. The MS parameters were as follows: a capillary voltage of 3.2 kV, ion source and solvation temperatures of 150°C and 350°C, respectively. Nitrogen was used as the nebulizer, desolvation, and sweep gas. Data acquisition was performed in multiple reaction monitoring (MRM) mode, thus ensuring sensitivity and selectivity of the method. Detailed information on the ion transitions used for each of the mycotoxins is described in Monbaliu et al. (2010). Data acquisition and processing were performed using MassLynx software (Micromass, Manchester, UK). The different steps of the analytical procedure are illustrated in **Figure 1**.

Figure 1:
Mycotoxin analytical procedure

Statistical Analysis

The ANOVA test was used, at a significance level of 0.05, to compare the means of mycotoxin concentrations in different substrates using SPSS Statistics 28.0 software.

RESULTS

Mycotoxin Occurrence in Samples

Figure 2 shows the occurrence of mycotoxins in the 18 samples tested, while **Figures 3, 4,** and **5** show the contaminants found in maize flour, cassava flour, and peanut paste samples, respectively.

Figure 2:
Occurrence of mycotoxins in the 18 samples tested

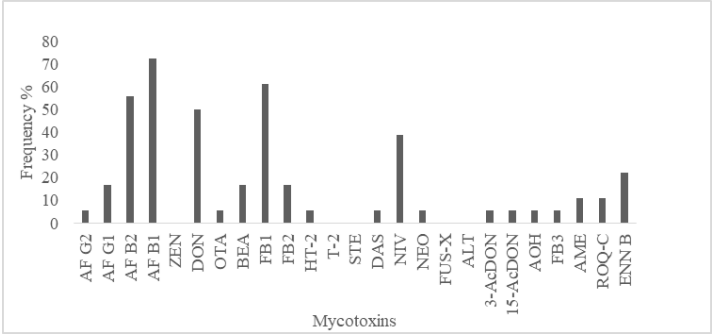


Figure 3:
Occurrence of mycotoxins in maize flour

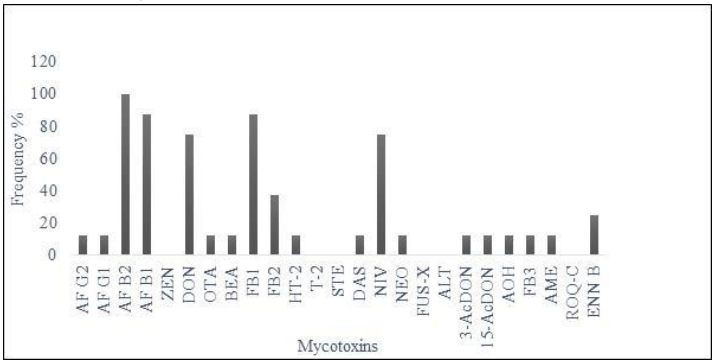


Figure 4:
Occurrence of mycotoxins in cassava flour

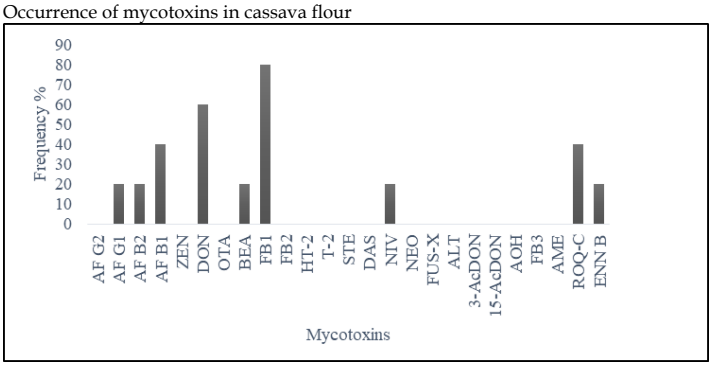
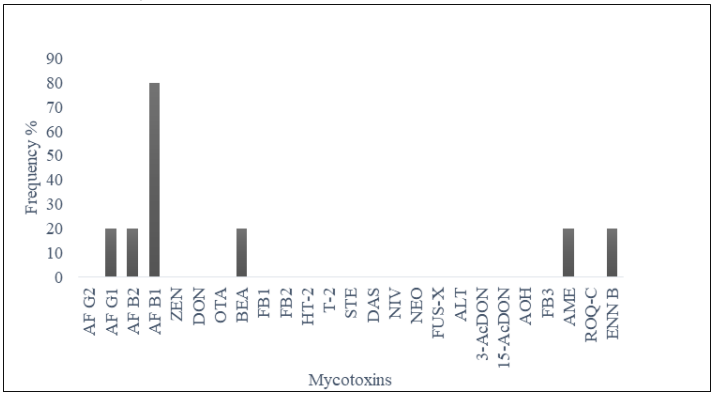


Figure 5:
Occurrence of mycotoxins in peanut paste



These **Figures** show that among the targeted Aspergillus mycotoxins, AFB1 was the most frequent contaminant in all the samples examined, appearing in very high proportions in maize flour and peanut paste. AFB2 was found in all maize flour samples, and OTA was identified in only one maize flour sample (**Figures 2, 3,** and **5**).

Regarding Fusarium mycotoxins, FB1 and DON were the most frequently encountered mycotoxins in the samples analyzed, found in high proportions in maize and cassava flours but not present in peanut paste (**Figures 2, 3, 4,** and **5**). In contrast, other regulated Fusarium mycotoxins (HT-2 and FB3) were detected at low frequencies in maize flour, the only staple food in which they were detected **(Figure 3)**.

As for the emerging mycotoxins, ENN B and BEA were the most frequently encountered metabolites in all foods analyzed (**Figures 3, 4,** and **5**). Maize flour was the only food type that contained all the emerging mycotoxins detected in this study **(Figure 3)**.

Data on the co-occurrence of the different mycotoxins in all analyzed samples are reported in **Table 1**. It can be noted that the AF/F combination was the most frequently observed in all the samples analyzed, and all the co-occurrences identified were more frequent in maize flour than in other staple foods.

Table 1:
Co-occurrences of AF/F, AF/OTA, AF/DON, and DON/F

Food	N	n'(F) AF/F	n'(F) AF/OTA	n'(F) AF/DON	n'(F) DON/F
Maize flour	8	7(87.5%)	1(12.5%)	6(75%)	5(62.5%)
Cassava flour	5	2(40%)	0(0%)	1(20%)	2(40%)
Peanut paste	5	0(0%)	0(0%)	0(0%)	0(0%)
Total	**18**	**9(50%)**	**1(5.6%)**	**7(38.8%)**	**7(38.8%)**

n: number of samples collected; n': number of samples contaminated with mycotoxin combinations; F: Frequency

Concerning the number of mycotoxins detected in the samples, the average number of mycotoxins detected per sample was 4, while in maize flour, cassava flour, and peanut paste, these were 6, 3, and 2, respectively. The maximum number of co-occurring mycotoxins was 4 in a maize flour sample.

Concentration Levels

The mycotoxin concentrations found in the investigated samples are presented in **Table 2** for maize flour, cassava flour, and peanut paste.

Table 2:
Mycotoxin concentrations in maize flour (n=8), cassava flour (n=5) and peanut paste (n=5)

Mycotoxins	Maize flour					Cassava flour					Peanut paste				
	EC regulatory limit µg/kg (EC 2023)	n'(P %)	Mean µg/kg	Interval µg/kg	n'' (P %)	EC regulatory limit µg/kg	n' (P %)	Mean µg/kg	Interval µg/kg	n'' (P%	EC regulatory limit µg/kg (EC 2023)	n' (P %)	Mean µg/kg	Interval µg/kg	n'' (P %)
AFB1	2	7(87.5)	2.2	1.2-5.9	2(25)	2	2(40)	0.2	0.1-0.3	-	2	4(80)	1.3	0.1-2.3	1(20)
AFB2	-	8(100)	1.1	1-1.8	-	-	1(20)	-	-	-	-	1(20)	0.9	0.9	-
AFG1	-	1(12.5)	2.2	2.2	-	-	1(20)	0.3	0.3	-	-	1(20)	3.4	3.4	-
AFG2	-	1(12.5)	1.9	1.9	-	-	1(20)	-	-	-	-	nd	nd	-	-
AFT	4	8(100)	3.6	1-7.7	2(25)	4	2(40)	0.35	0.1-0.6	-	4	4(80)	2.4	0.1-6.6	1(20)
OTA	3	1(12.5)	5.2	5.2	1(12.5)	3	nd	nd	-	-	3	nd	nd	-	-
FB1	-	7(87.5)	14.5	1.8-39.2	-	-	4(80)	8.9	2.1-16.4	-	-	nd	nd	-	-
FB2	-	3(37.5)	22	19.1-25.1	-	-	nd	nd	-	-	-	nd	nd	-	-
FB1+FB2	1000	7(87.5)	23.9	1.8-64.3	-	1000	4(80)	8.9	2.1-16.4	-	1000	-	-	-	-
DON	750	6(75)	4.5	2.3-7.3	-	750	3(60)	1	0.1-2	-	-	nd	nd	-	-
NIV	-	6(75)	13.9	12.3-16.1	-	-	1(20)	1	1	-	-	nd	nd	-	-
BEA	-	1(12.5)	20.6	20.6	-	-	1(20)	2.6	2.6	-	-	1(20)	225.4	225.4	-
ENN B	-	2(25)	7.6	7.6	-	-	1(20)	0.2	0.2	-	-	1(20)	0.3	0.3	-
AOH	-	1(12.5)	4.5	4.5	-	-	nd	nd	-	-	-	nd	nd	-	-
AME	-	1(12.5)	19.9	19.9	-	-	nd	nd	-	-	-	1(20)	9	9	-
DAS	-	112.5)	0.4	0.4	-	-	nd	nd	-	-	-	nd	nd	-	-
NEO	-	1(12.5)	4.1	4.1	-	-	nd	nd	-	-	-	nd	nd	-	-
3-AcDON	-	1(12.5)	0.8	0.8	-	-	nd	nd	-	-	-	nd	nd	-	-
15-AcDON	-	1(12.5)	1.2	1.2	-	-	nd	nd	-	-	-	nd	nd	-	-
FB3	-	1(12.5)	26.1	26.1	-	-	nd	nd	-	-	-	nd	nd	-	-
HT-2	-	1(12.5)	7.2	7.2	-	-	nd	nd	-	-	-	nd	nd	-	-
STE	-	nd	nd	-	-	-	nd	nd	-	-	-	nd	nd	-	-
FUS-X	-	nd	nd	-	-	-	nd	nd	-	-	-	nd	nd	-	-
ROQ-C	-	nd	nd	-	-	-	2(40)	0.1	0.1	-	-	nd	nd	-	-
ZEN	75	nd	nd	-	-	-	nd	nd	-	-	-	nd	nd	-	-
T-2	-	nd	nd	-	-	-	nd	nd	-	-	-	nd	nd	-	-
ALT	-	nd	nd	-	-	-	nd	nd	-	-	-	nd	nd	-	-

n': number of positive samples, n'': number of non-compliant samples, P: proportion, nd: not detected, -: not relevant

This Table shows that, for Aspergillus mycotoxins, 25% of maize flour samples and 20% of peanut paste samples had AFB1 concentrations above the European maximum level (Table 2), with much lower levels in cassava flour (Table 2). Additionally, 25% of maize flour samples and 20% of peanut paste samples were above the EU regulatory limits for AFT **(Table 2)**. Only one maize flour sample was contaminated by OTA with a concentration higher than the corresponding EU regulatory limits **(Table 2)**.

Regarding Fusarium mycotoxins, although low, the concentrations of FB1, total fumonisin, and DON were higher in maize flour than in cassava flour without exceeding the limits of European regulation **(Table 2)**. As for the emerging mycotoxins, the BEA concentration was higher in peanut paste (225.4 µg/kg), unlike other mycotoxins whose concentrations were higher in maize flour **(Table 2)**.

Table 3 compares the average concentrations of AFB1 and AFT in the studied substrates.

Table 3:
Comparison of the average concentrations of AFB1 and AFT in the different substrates

Mycotoxins	Substrates	P-value
AFB1	Maize flour/cassava flour	0.0023
	Peanut paste/cassava flour	0.022
	Maize flour/peanut paste	0.107
AFT	Maize flour/cassava flour	0.019
	Peanut paste/cassava flour	0.030
	Maize flour/peanut paste	0.086

The analyses in this table show statistically significant differences for all comparisons except those between maize flour and peanut paste.

DISCUSSION

The finding that more than 80% of maize flour and peanut paste samples were contaminated with AFB1 confirms the predilection of these matrices for the development of *Aspergillus flavus*. These results are comparable to those obtained by Mulunda et al. (2013), who reported more than 90% AFB1 contamination in maize and peanut samples collected in Lubumbashi, as well as those highlighted by Udomkum et al. (2018) on samples of maize and peanut paste collected in North Kivu (Kabare

and Uvira). Manizan et al. (2018) also noted more than 95% AFB1 contamination in samples of peanut paste and maize flour in their multi-mycotoxin study on cereals and oilseeds consumed in Ivory Coast. The presence of aflatoxins in maize flour and peanut paste could be attributed to the hot and humid climate, inappropriate agricultural practices, and poor storage conditions, as reported by Kamika et al. (2014). In this study, the EU limits for AFB1 and AFT (EC, 2023) were exceeded only for 25% of samples for maize flour and 20% for peanut paste. In contrast, in other studies carried out in the DRC (Udomkun et al., 2018) and elsewhere in Africa (Manizan et al., 2018; Meijer et al., 2021), the proportions of samples exceeding the regulatory levels were well above 50%. These large discrepancies could be explained in particular by the sampling period and the respect of hygiene conditions from farm to fork (Kamika et al., 2016; Nji et al., 2022b). Furthermore, the low rates of samples exceeding the maximum European limit revealed in this study could be explained by greater compliance with sorting conditions before grinding, as suggested by Nji et al. (2022b).

Low concentrations of aflatoxins in cassava flour were observed in the present study, as in other studies carried out in the DRC (Udomkun et al., 2018) and in other countries of the African continent (Chiona et al., 2014; Obong'o et al., 2020; Sulyok et al., 2015; Abass et al., 2017). This is most probably due to the fact that during the fermentation process leading to the production of cassava flour, lactic acid bacteria and other strains such as Saccharomyces cerevisiae degrade aflatoxins (Ahlberg et al., 2015; Nji et al., 2022b). These low concentrations of AFs could also be explained by the poverty of cassava in triglycerides, substrates involved, when simple sugars are exhausted, both in the development of *Aspergillus flavus* and in the production of AFs. Indeed, according to the results of the work carried out on cotton by Mellon et al. (2000), lipids influence the production of AFs. They found that when triglycerides were extracted from the medium under study, the production of AFs decreased more than 800 times. Using kinetic, growth probability, and detection time models, Kosegarten et al. (2016) also demonstrated that the growth of *Aspergillus flavus* was influenced by various factors, in particular the presence of triglycerides. Moreover, statistical analysis using ANOVA to compare the mean concentrations of AFB1 and AFT between cassava flour and the other two substrates revealed statistically significant differences ($p<0.05$) **(Table 3)**. Conversely, it only needed to be enriched with the same substrate to restore it.

The results on OTA contamination (12.5% of maize flour samples) are in agreement with those of Kouadio et al. (2014) obtained in Ivory Coast on 13% of maize flour samples. The presence of these mycotoxins in certain samples exceeding the maximum European limit can cause toxic effects including carcinogenicity, estrogenicity, neurotoxicity, genotoxicity, nephrotoxicity, immunotoxicity, and hepatotoxicity (Ekwomadu et al., 2021; Khan et al., 2024). The present study reveals the presence of DON, FB1, and NIV, Fusarium mycotoxins, at high frequencies in maize and cassava flours. This confirms the results of the BIOMIN Mycotoxin Survey (2021), which states that these contaminants are increasingly encountered in sub-Saharan Africa. Mulunda et al. (2013) in the DRC and Hanvi et al. (2019) in Togo made the same observations for maize flour samples. The conspicuous presence of mycotoxins in food could result in an increased risk of acute and chronic intoxications in the concerned population. Indeed, FB1 could increase the risk of esophageal cancers and neural tube defects in humans (Manizan et al., 2018; Ekwomadu et al., 2020); DON could be the cause of several non-specific symptoms such as vomiting and diarrhea (Ekwomadu et al., 2020), while NIV may cause immunosuppressive and protein inhibitory effects (Ekwomadu et al., 2020). In this study, the concentrations of FB1 (1.8-39.2 µg/kg), the sum of FB1 and FB2, and DON are higher in maize samples than in cassava flour (2.1-16.4 µg/kg for FB1) but at very low levels not exceeding EU regulatory limits (EC, 2023). Low concentrations of FB1 were also reported in Ivory Coast by Sangare et al. (2007) (0.3-1.5 µg/kg) in maize flour as well as by Ediage et al. (2011) (4-21 µg/kg) in cassava flour from Benin.

As for the presence of emerging mycotoxins, the results obtained in this study, for both BEA in maize flour samples (12.5%) and ENN B in peanut paste (20%), are very close to those reported by Manizan et al. (2018) (11% and 18%), but higher than those reported by Manizan et al. (2018) and Warth et al. (2012), which are less than 12 and 3

µg/kg respectively for BEA and ENN B. In addition, the presence of AME and AOH, as in the case in the present work, has also been reported in maize samples in Burkina Faso and Mozambique (Warth et al., 2012). Particular attention should therefore be paid to this category of mycotoxins in view of their toxicity and their probable synergy following the co-occurrence of other regulated mycotoxins (Smith et al., 2016; Gruber-Dorninger et al., 2017). With regard to co-occurrence, the results obtained in this study are in agreement with those observed by Smith et al. (2016) in particular for the AF/F combination, which is characteristic of the African continent alongside other associations such as AF/OTA, AF/ZEN, and AF/OTA/ZEN. Given that DON and fumonisins have become the most widespread mycotoxins in the world in general and particularly in sub-Saharan Africa (BIOMIN Mycotoxin Survey, 2021), it is more than obvious that binomials such as AF/F, AF/DON, and DON/F are frequently observed as in the present study in maize flour samples.

CONCLUSION

To conclude, the multi-mycotoxin analysis implemented in this study allowed the detection and quantification of 20 mycotoxins out of the 25 targeted in the samples of maize flour, cassava flour, and peanut paste collected in different markets of Kinshasa. The results showed that the maize flour samples were the most contaminated with major (AFB1, OTA, F, DON, NIV) and emerging (BEA, ENN, AOH, AME) mycotoxins and therefore the most concerned by co-occurrences (AF/F, AF/DON, DON/F, and AF/OTA). The study also revealed that for some maize flour and peanut paste samples, AFB1, AFT, and OTA concentrations were above the maximum limits laid down by the European Commission. The regulatory limits set for the total fumonisin were not exceeded in any of the analyzed samples. The results of the present study indicate that maize flour and peanut paste, compared to cassava flour, are the staple foods that would account the most for the exposure of the Kinshasa population to mycotoxins. This exposure could have significant public health implications due to the potential toxic effects of mycotoxins, which include carcinogenicity, immunotoxicity, hepatotoxicity, and neurotoxicity.

The study's findings highlight the necessity for increased awareness and implementation of mycotoxin control measures, particularly in maize flour and peanut paste. Improving agricultural practices, storage conditions, and regular monitoring of mycotoxin levels in food products can help mitigate these risks. Additionally, the results underline the importance of strict adherence to regulatory limits to safeguard public health.

Recommendations

Based on the findings, several recommendations can be made:

1. **Enhanced Monitoring and Control:** There should be regular monitoring of mycotoxin levels in food products, especially maize flour and peanut paste, to ensure they are within safe limits. Authorities should enforce strict control measures to minimize contamination during pre-harvest, harvest, and post-harvest stages.
2. **Improved Agricultural Practices:** Farmers should be educated on best agricultural practices to reduce mycotoxin contamination. This includes proper crop rotation, timely harvesting, and the use of resistant crop varieties.
3. **Better Storage Facilities:** Investment in better storage facilities that control humidity and temperature can significantly reduce the risk of mycotoxin contamination.
4. **Public Awareness Campaigns:** There should be ongoing public awareness campaigns to educate the population about the risks of mycotoxin contamination and the importance of consuming safe, regulated food products.
5. **Research and Development:** Further research should be conducted to develop effective and practical methods for mycotoxin decontamination and to explore the potential health impacts of long-term exposure to multiple mycotoxins.

By implementing these measures, it is possible to significantly reduce the risk of mycotoxin contamination in staple foods and protect the health of the Kinshasa population.

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PBM performed data analysis; MKK, ADM, JMK, PBM, CMT, SDS, and JDDM wrote the manuscript.

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