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Prevalence of Mycotoxins in Raw Materials and Infant Flours Produced in Burkina Faso

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Abstract: Mycotoxins are toxic secondary metabolites secreted mainly by molds. The contamination of the raw materials used in the production of infant flours by these mycotoxins presents a risk to the health of infants and young children who are the main consumers of these flours. The objective of this study was to determine the prevalence of mycotoxins in raw materials and in infant flours from Burkina Faso. Analyses were carried out on 39 samples of raw materials and 26 samples of infant flour collected from artisanal and semi-industrial production units. The contents of AFB1, AFB2, AFG1, AFG2, FB1 and OTA were determined by LCMS/MS. The results indicated a variability in the contents of total aflatoxins (AFs), FB_1 and OTA within the samples of raw materials. Also, 79.49% of the raw materials contaminated by total aflatoxins were above the maximum limit value and 61.54% of these raw materials had AFB_1 levels above the recommended limit value. The highest levels of AFs were found in peanuts and maize. OTA was very present in soybean samples. The occurrence of mycotoxins was highly variable in infant flours within the same production unit ($p < 0.001$). and between different units ($p < 0.001$). Infant flours produced by artisanal production units had higher AFB_1 contents than those produced by semi-industrial units. The need to control these mycotoxins is essential in order to considerably reduce their contamination and exposure in infants and young children and to preserve their health.

Keywords: raw materials; infant flours; mycotoxins; occurrence; Burkina Faso

1. Introduction

Secreted by microscopic fungi mycotoxins are toxic secondary metabolites. They represent a potential health risk to humans and animals. They are best known for the intoxications they can induce after consumption of contaminated food and the problems they cause for food industry and health [1]. Most mycotoxins are suspected to be carcinogenic, and are low molecular weight compounds that are non-volatile at ambient temperatures [2]. Aflatoxins (AFs), Ochratoxin A (OTA) and Fumonisins are among the mycotoxins considered to be the most important in the food and health sector [3,4]. Adverse effects of mycotoxins observed in humans and animals include carcinogenicity, teratogenicity, immunogenicity, neurotoxicity, hepatotoxicity, nephrotoxicity, reproductive and developmental toxicity among others [5]. Previous studies have shown that cereals such as maize, millet, sorghum, rice and leguminous such as peanut were contaminated by mycotoxins in west Africa including Burkina Faso [6–13]. These contaminations can occur either in the field, during harvesting or storage [14,15]. Mycotoxins are major risk factors in human and animal nutrition [16] The raw materials mainly used in the production of infant flours in Burkina Faso are

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cereals (millet, maize, sorghum, rice) and legumes (peanut, soybeans, cowpeas). Studies conducted in Burkina Faso have shown that infant flours presented high levels of mycotoxins and that the frequency of contaminated samples by aflatoxin B1 (AFB1), Ochratoxin A and fumonisins were 83.9%, 7.5% and 1.5%, respectively [11]. Yet, no investigation about the occurrence of mycotoxins in raw materials intended for infant flours has been conducted to date in Burkina Faso. The main objective of this study was to determine the prevalence of mycotoxins in raw materials intended for infant fours' production as well as infant flour themselves produced in Burkina Faso. More specifically, it was to determine the occurrence of AFs, fumonisin B1 (FB1) and OTA in these raw materials and infant flours. The production of infant flours destined for wean age children is more and more of great importance to combat undernutrition in developing countries. Then the study on the occurrence of these mycotoxins in the different samples of infant flours from different and types of production units will contribute in the safety management of food intended for this vulnerable group.

2. Materials and Methods

2.1. Study Design

This was a cross-sectional study including all the infant flour production units identified in the city of Ouagadougou. The different raw materials and infant flours used for this study were based from 11 infant flour production units investigated. The samples were collected from July to September 2021. In total, 39 samples of raw materials, and 26 infant flour samples have been collected. Most infant flour were packaged in 500 g packages. For each sample, 500g were taken.

2.2. Sample Collection

The samples were collected in the city of Ouagadougou, capital of Burkina Faso. The raw materials collected were millet, maize, sorghum, rice, peanut, cowpeas and soybeans. Infant flour samples were collected within the production units either free of charge or after payment. Infant flours were packaged in 500 g or 1 Kg. A total of 26 flour samples were collected in artisanal and semi-industrial production units. Artisanal production units are production units that utilize simple equipment such as basins, sieves, roasters, mixers and bag welding machines. The artisanal production method requires a large workforce. However, semi-industrial production units generally use mechanised production equipment, with recourse to manual processes for certain stages. Most artisanal production units were supplied with raw materials directly from the markets while semi-industrial production units had storage stores and approved suppliers. The collection of raw material samples intended for infant flour production took place in the storage warehouses of the production units. For the infant flour production units that obtain their supplies directly from the markets, the raw materials were collected from the traders of the markets concerned. The square root of each stock sample was collected and sub samples of 5 Kg were derived for analysis. A total of 39 samples of raw materials were collected, including 22 samples of cereals (millet, maize, sorghum, rice) and 17 samples of legumes (peanuts, soybeans, cowpeas).

2.3. Sub-Sampling of Raw Materials

Each raw material sample was divided equally into 3 subsamples using a sub-sampler mill VSLIC-A110. The 2 sub-samples were kept and the other was ground using a grinder for analysis. The cereal samples (rice, sorghum, maize, millet) have been ground in the period from October 17 to 20, 2022 and the legumes samples (cowpea, soya, peanut) have been ground from November 2 to 5, 2022. The grinder has been thoroughly cleaned after each grinding with carded Cotton and methanol to avoid any cross-contamination.

2.4. Sample Preparation

The modified QuEChERS method described by Amirahmadi and *al.* [17] has been used. Briefly, 5 g of each sample was weighed into 50 ml tube and 25 ml of the extraction solution (Water/Formic

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acid/Acetonitrile 20/1/70) and 1 g of NaCl was added respectively. The mixture was stirred for 10 minutes using a magnetic stirrer MH 15. Then 5 g of magnesium sulphate was added to the mixture and mixed with a vortex for 2 minutes then centrifuged for 10 minutes at 1500 G.

After centrifugation, 5 ml of the supernatants were taken into 15 ml tubes and 1 g of magnesium sulphate and 0.3 g of Primary Secondary Amine (PSA) were added. The mixture was then vortexed for 2 minutes and centrifuged for 10 minutes at 1500 G. After centrifugation, 4 ml of the extracts were taken and evaporated to dryness. The solutions were then reconstituted with 1 ml of methanol and placed in Vials for LC-MS/MS analysis.

2.5. LC-MS/MS Analysis

A The LC-MS/MS Agilent Technology 1290 Series coupled with mass spectrometer Agilent Technology 6430 was used to perform the analysis. The separation was carried out by a Zorbax Eclipse XDB C18 column (50 x 4.6 mm, 1.8 µm) (Agilent, USA) using water (0.1% formic acid, 5 mM ammonium formate) as mobile phase A and methanol (0.1% formic acid, 5 mM ammonium formate) as mobile phase B. The gradient elution was set as follow: 10% B from 0.01 to 0.5 min, 10–50% B from 0.5 to 10 min, 100% from 10–11 min, 10% 11–13.5 and 0% B from 13.5 to 16 min. The flow rate was maintained at 0.5 ml/min.

The Multi-Reaction Monitoring (MRM) scanning and electro-spray ionisation on positive mode were use. The precursor and product ions are presented in Table 1

Table 1. Precursor and product ions.

Mycotoxins	Precursor ion (M/Z)	Qualifier ion (M/Z)	Quantifier ion (M/Z)
Aflatoxin B1	313	285.1	241.0
Aflatoxin B2	315	287.1	259.1
Aflatoxin G1	329	233	311
Aflatoxin G2	331	313	245
Fumonisin B1	722.2	334.2	252.2
OTA	404	239	221

2.6. Quality Assurance and Quality Control

The analytical method was validated according to SANTE/ 11945/2015 directives. The quality control material (TQC-MT100) provided by Trilogy Analytical Laboratory (USA) was used. The limits of detection were ranged from 3 to 502 ng/Kg (AFB1= 5; AFB2= 3; AFG1= 14; AFG2= 9; FB1= 91 and OTA= 502). The limits of quantification were ranged from 9 to 1675 ng/Kg (AFB1= 15; AFB2= 9; AFG1= 47; AFG2= 30; FB1= 302 et OTA= 1675) and lastly, the recovery rates were 91.30% for AFB1; 92% for AFB2, AFG1 and AFG2; 88.40% for FB1 and 85.20% for OTA.

2.7. Statistical Analysis

The analysis of the mycotoxin content in the different samples was carried out in triplicate. Excel 2019 and STATA 16.0 software were used for data analysis. The different parameters were compared using the comparison test of proportions and the comparison test of averages at 5% level.

3. Results

3.1. Occurrence of Mycotoxins in Raw Materials

Table 2 shows contamination of raw materials by AFs, FB1 and OTA. Results indicated that all raw materials were contaminated with aflatoxins. We had 20.51% of the raw materials that had AFs contents < 4 µg/Kg, 20.51% had contents between [4–10[µg/Kg and 58.98% had contents ≥10 µg/Kg. The raw materials containing the high levels of Total aflatoxins (AFs) were peanut and maize with means of 46.67 and 41.37 µg/kg respectively for peanut and maize. Cowpea, rice and soybean had the lowest AFs contents (i.e. Mean = 8.75 µg/kg for cowpea, 6.86 µg/kg for rice and 6.95 µg/kg for soybean). The comparison of averages indicates a significant difference between the AFs contents within the same types of raw material samples and between the different groups of raw materials ($p < 0.001$). We had 79.49% of raw materials that were not contaminated by FB1 and 20.51% that had FB1 contents below the maximum authorised limit [18]. No sample of sorghum, maize, rice or cowpea contained fumonisin B1. On the other hand, fumonisin B1 was detected in millet at the minimum level of 9.68 µg/kg to the maximum level of 58.44 µg/kg. In soybean, FB1 content varied from 6.30 µg/kg to 58.40µg/kg and in peanut from 7.28 µg/kg to 7.79 µg/kg. The comparison of averages indicated a significant difference between the FB1 contents within the same type of raw material samples ($p < 0.001$) and between the different groups of raw materials ($p < 0.001$). Results indicated that 61.54% of raw materials were not contaminated by OTA, 7.69% of contaminated raw materials were below the Limit Value set by the European Commission (5µg/Kg) [18] against 30.77 % that were above the limit (5.13% were between [3-5 µg/Kg] and 25.64% ≥10 µg/Kg). The level of OTA contamination was ranged from 0.51 µg/kg in maize to 56.49 µg/kg in cowpea. The contamination was high in the soybean samples with a mean of 32.10 ±5.91 µg/kg. The contamination level was moderately high in peanut (Mean = 14.59 ± 5.39 µg/kg), rice (Mean = 5.09±2.10 µg/kg) and maize (Mean = 0.51±0.01 µg/kg). OTA contents were absent in millet, red sorghum and white sorghum samples. The comparison of averages indicates a significant difference between the OTA contents within the same types of raw material samples ($p < 0.001$) and between the different groups of raw materials ($p < 0.001$).

Table 2. Contents (µg/kg) of mycotoxins in raw materials (mean±STD).

Sample level	AFB1	AFB2	AFG1	AFG2	AFs	FB1	OTA
Mi 6	1.54±0.44	0.76±0.21	8.04±0.92	3.77±0.58	14.10±2.15	9.68±16.76	0.00
Mi MSL	1.38±1.37	0.81±0.36	3.62±2.13	4.91±1.31	10.72±5.18	58.44±101.21	0.00
Mi MRJ	6.69±1.08	2.18±0.34	6.69±2.10	11.50±1.73	27.06±5.24	0.00	0.00
Mi BSR	22.37±1.35	2.07±0.91	2.07±1.01	4.31±1.18	30.81±4.45	0.00	0.00
Mi KSN	15.31±1.91	0.77±0.15	2.61±1.56	3.93±2.03	22.62±5.65	0.00	0.00
Mi BAM	41.21±4.55	3.13±0.24	6.58±4.00	3.87±2.38	54.80±11.17	0.00	0.00
Mi SDP	3.87±1.25	1.54±1.33	5.80±0.79	10.98±2.47	22.18±5.84	0.00	0.00
So BSR	12.04±0.84	6.35±0.34	1.71±0.94	1.80±1.13	21.90±3.25	0.00	0.00
So MRJ	1.40±0.10	0.34±0.58	1.96±0.98	0.52±0.09	4.22±1.76	0.00	0.00
So 6	6.67±0.25	2.87±0.15	2.20±1.48	2.55±0.46	14.29±2.34	0.00	0.00
Ri 6	3.69±1.38	0.40±0.49	0.70±0.66	1.64±1.18	6.43±3.71	0.00	5.79±1.22
Ri RSL	3.37±0.15	1.02±0.57	2.32±1.40	1.76±0.53	8.47±2.65	0.00	6.75±1.24
Ri MRJ	1.51±0.53	0.26±0.25	2.53±0.37	2.24±0.91	6.54±2.05	0.00	2.74±1.33
Ri SC	2.46±1.00	0.49±0.44	1.23±1.53	1.84±0.85	6.02±3.82	0.00	0.00
Ma G	1.26±0.24	0.22±0.20	1.71±1.32	0.50±0.53	3.69±2.28	0.00	0.00
Ma FTZ	1.96±0.35	0.78±0.73	0.25±1.32	0.28±0.49	3.27±1.84	0.00	0.00
Ma RSL	64.36±8.40	12.12±0.36	1.15±0.53	1.37±1.19	79.00±10.47	0.00	0.52±0.89
Ma 6	1.76±0.11	0.30±0.27	0.45±0.79	0.00	2.51±1.16	0.00	0.00
Ma BSR	69.56±8.95	13.87±0.84	2.82±0.62	1.53±0.37	87.78±10.78	0.00	0.00
Ma SDP	24.67±1.36	6.35±0.53	1.96±0.97	1.37±0.82	34.35±3.69	0.00	0.00
SJ KSN	6.93±0.77	1.46±0.56	0.85±0.81	0.80±0.54	10.05±2.68	58.40±34.15	0.00
SJ SDP	0.68±0.60	0.51±0.13	0.59±0.20	0.56±0.48	2.34±1.41	0.00	21.77±18.85
SJ G	0.67±0.12	0.30±0.37	1.35±0.61	1.66±0.74	3.97±1.84	0.00	34.42±1.99
SJ FTZ	1.15±1.98	0.44±0.39	2.15±2.68	10.94±18.16	14.68±23.22	0.00	34.17±34.72
SJ MSL	0.39±0.35	0.71±0.12	1.04±0.95	1.64±1.06	3.78±2.49	6.30±10.92	36.73±4.22
SJ BAM	2.00±1.79	0.19±0.17	0.84±0.33	0.76±0.38	3.79±2.66	0.00	33.43±2.08
N FTZ	0.60±0.59	1.23±0.35	3.25±2.70	5.82±1.53	10.89±5.18	0.00	56.49±19.06
H MRJ	4.29±0.99	0.32±0.29	0.51±0.45	1.49±1.08	6.61±2.81	0.00	0.00
Ar BAM	0.00	0.36±0.39	0.79±0.38	1.34±0.72	2.50±1.49	0.00	12.75±22.08
Ar MSL	1.38±0.32	0.54±0.19	1.16±0.65	2.79±0.97	5.87±2.13	7.79±13.49	22.59±19.64
Ar G	101.96±2.90	22.52±0.84	8.78±1.08	6.72±1.89	139.99±6.72	0.00	12.08±20.91
Ar BSR	24.07±0.72	3.34±0.72	0.76±0.66	1.38±0.89	29.55±2.99	0.00	0.00
Ar FTZ	33.96±6.36	7.96±0.59	0.77±0.80	1.54±0.39	44.23±8.14	0.00	0.00
Ar KSN	52.36±4.33	13.51±1.03	2.81±1.56	3.48±1.73	72.16±8.65	7.28±12.61	0.00
Ar MRJ	3.62±0.36	0.64±0.12	1.14±0.58	1.54±1.48	6.94±2.55	0.00	10.94±18.95

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Mi CRL	15.31±1.91	0.77±0.15	2.61±1.56	3.93±2.03	22.62±5.65	0.00	0.00
Ma CRL	64.36±8.40	12.12±0.36	1.15±0.53	1.37±1.19	79.00±10.47	0.00	0.51±0.89
SJ CRL	6.93±0.77	1.46±0.56	0.85±0.81	0.80±0.54	10.05±2.68	58.40±34.15	0.00
Ar CRL	52.36±4.33	13.51±1.03	2.81±1.56	3.48±1.73	72.16±8.65	7.28±12.61	0.00

3.2. Occurrence of Mycotoxins in Infant Flours

Table 3 shows that all samples were contaminated by AFs. The contamination level was high in infant flours containing mainly peanut meal. The results also indicated that 40.31% samples of infant flour were not contaminated by FB1 and that 53.85% had lower contents than 200 µg/Kg. Only 3.85% had contents greater than 200 µg/Kg. the FB1 content varied from 1.53 µg/kg to 417.09 µg/kg. We had 38.46% of infant flour samples that were not contaminated by OTA, 57.69% that had contents lower than 3 µg/Kg and 3.85% that had contents greater than 10 µg/Kg. The contamination of OTA was ranged from 0.23 µg/kg to 37.43 µg/kg.

Table 3. mycotoxin contents in infant flours (mean ± STD).

Infant flours	AFB1	AFB2	AFG1	AFG2	AFs	FB1	OTA
FSo6	2.40±1.69	1.98±0.42	21.91±6.81	2.57±1.78	28.87±10.70	82.50±27.90	2.88±2.62
BRPE1	5.36±0.30	2.71±0.24	11.82±3.41	1.58±0.33	21.47±4.28	37.48±3.89	2.80±1.03
Fri	2.58±0.84	0.51±0.24	3.66±0.85	1.75±1.49	8.51±3.42	32.60±11.56	0.36±0.62
BRPE4	8.77±0.81	1.16±0.31	9.38±3.99	1.84±0.64	21.15±5.76	28.33±7.67	1.87±0.63
FMa6	1.75±0.33	1.12±0.24	4.40±2.32	1.52±0.93	8.78±3.82	27.68±7.68	1.94±0.62
BRPE2	1.33±0.44	1.35±0.26	2.76±1.87	2.55±2.52	7.99±5.09	27.21±26.26	0.84±0.75
BRPE3	3.97±0.57	3.42±0.66	3.60±1.87	3.61±1.88	14.59±4.97	4.98±8.63	0.00
FSoMRJ	2.27±0.33	1.49±0.87	12.09±3.76	3.90±1.79	19.74±6.74	2.57±4.45	2.42±4.19
VCS Inst	6.54±0.34	4.41±0.47	2.23±1.27	2.84±1.51	16.03±3.59	0.00	0.00
VTL Inst	0.59±0.38	0.84±0.52	0.84±0.40	0.55±0.32	2.82±1.62	0.00	0.00
FRi6	3.65±0.62	0.42±0.16	5.51±2.35	1.62±1.33	11.20±4.46	20.45±6.79	1.85±0.23
CRL	0.85±0.38	0.79±0.49	5.29±3.66	1.84±0.65	8.76±5.17	0.00	2.65±0.34
VTL Lc	1.37±0.37	1.46±0.33	8.12±6.66	2.27±1.11	13.22±8.48	0.00	0.00
FTZ	17.19±0.81	6.81±0.86	7.99±0.82	3.10±0.85	35.09±3.33	0.00	1.06±0.95
VCS Lc	0.68±0.19	0.65±0.24	4.34±1.07	1.87±1.80	7.54±3.31	0.00	0.26±0.45
NTV	5.27±0.20	1.29±0.79	5.71±2.57	4.56±3.29	16.84±6.86	1.53±2.66	0.00
FMi MRJ	2.17±0.36	2.73±1.54	3.42±1.66	7.89±5.02	16.22±8.58	0.00	0.00
SL	2.59±0.28	1.08±0.47	2.35±2.10	1.79±1.94	7.81±4.78	1.73±3.00	0.00
FMi 6	5.76±0.74	1.63±1.48	3.47±1.17	9.22±12.93	20.07±16.31	0.00	0.00
MSL	1.05±0.61	1.20±0.80	7.13±2.16	1.84±0.43	11.22±4.00	0.00	0.33±0.57
BAM	26.87±4.70	3.91±0.69	6.59±1.60	3.63±2.30	41.00±9.29	3.16±5.47	0.23±0.40
FH MRJ	1.59±0.34	0.51±0.24	2.38±0.97	2.83±0.29	7.31±1.84	8.80±0.54	0.00
FRi MRJ	0.75±0.29	0.37±0.32	5.44±1.95	0.96±1.19	7.53±3.76	2.64±4.57	0.88±0.76
KSN	0.89±0.12	1.17±0.63	5.09±1.19	1.85±0.93	8.99±2.87	0.00	0.00
PG Ma	2.89±0.56	2.27±0.51	1.73±0.86	1.50±1.43	8.40±3.36	0.00	0.24±0.41
Tar MRJ	62.88±4.49	17.17±1.41	16.63±7.11	6.74±3.65	103.43±16.66	417.09±117.15	37.43±8.45

3.3. Occurrence of AFB1 in Raw Materials according to the Types of Production Units

Figure 1 highlights the occurrence of AFB1 in raw materials according to the types of production units. The results indicate that the AFB1 contents in the samples of raw materials collected from artisanal units were high compared to the samples collected from semi-industrial units. At the level of artisanal units, the average AFB1 contents were 14.75 ± 15.34 µg/Kg in millet, 6.71 ± 5.32 µg/Kg in sorghum, 2.76 ± 0.98 µg/Kg in rice, 45.23 ± 37.73 µg/Kg in maize, 4.29 ±0.00 in cowpea, 3.11 ± 3.41 µg/Kg in soybeans and 39.29 ± 37.97 µg/Kg in peanut. As for semi-industrial units, the mean content of AFB1 was 9.59 ± 8.09 µg/Kg in millet, 23.06 ± 29.60 µg/Kg in maize, 0.60 ±0.00 in cowpea, 2.36 ± 3.06 µg/Kg soybeans and 33.96 ± 00.00 µg/Kg in peanut. The comparison of the mean contents in AFB1 in the two types of units showed a significant difference ($p = 0.013$).

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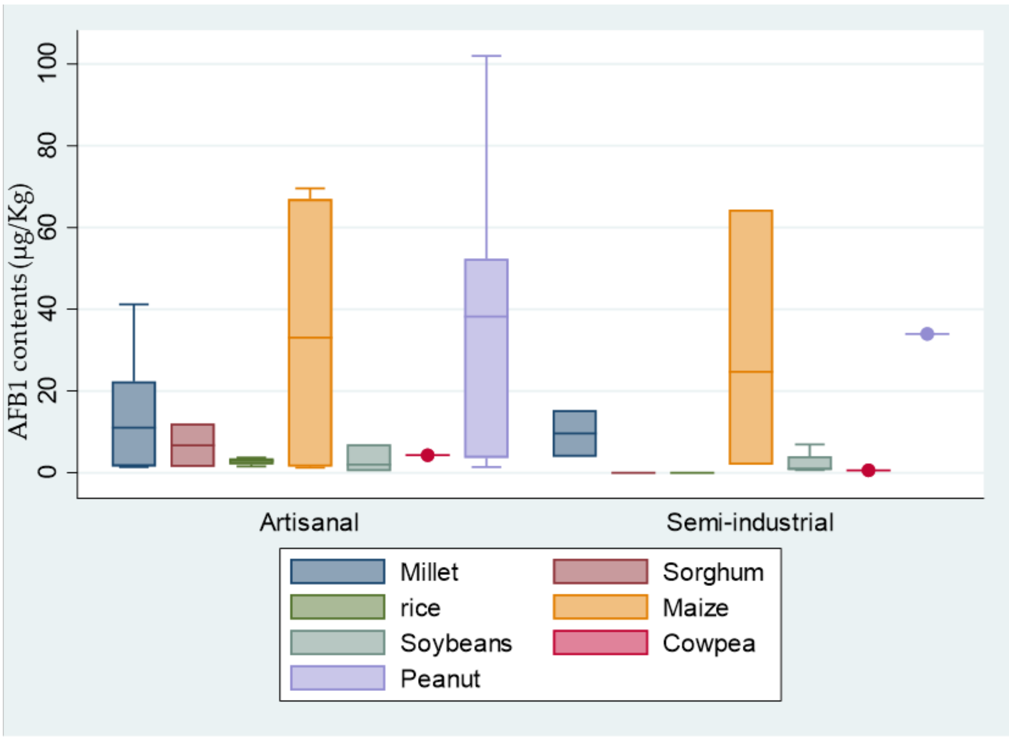

Figure 1. Occurrence of AFB1 in raw materials according to the types of production units.

3.4. Occurrence of AFB1 in Infant Flour according to The Production Units

Figure 2 highlights the occurrence of AFB1 in infant flour according to the types of production units. It appears that UP6 had the most contaminated infant flour in AFB1 (62.88µg/Kg) followed by UP2 (26.87 µg/Kg).

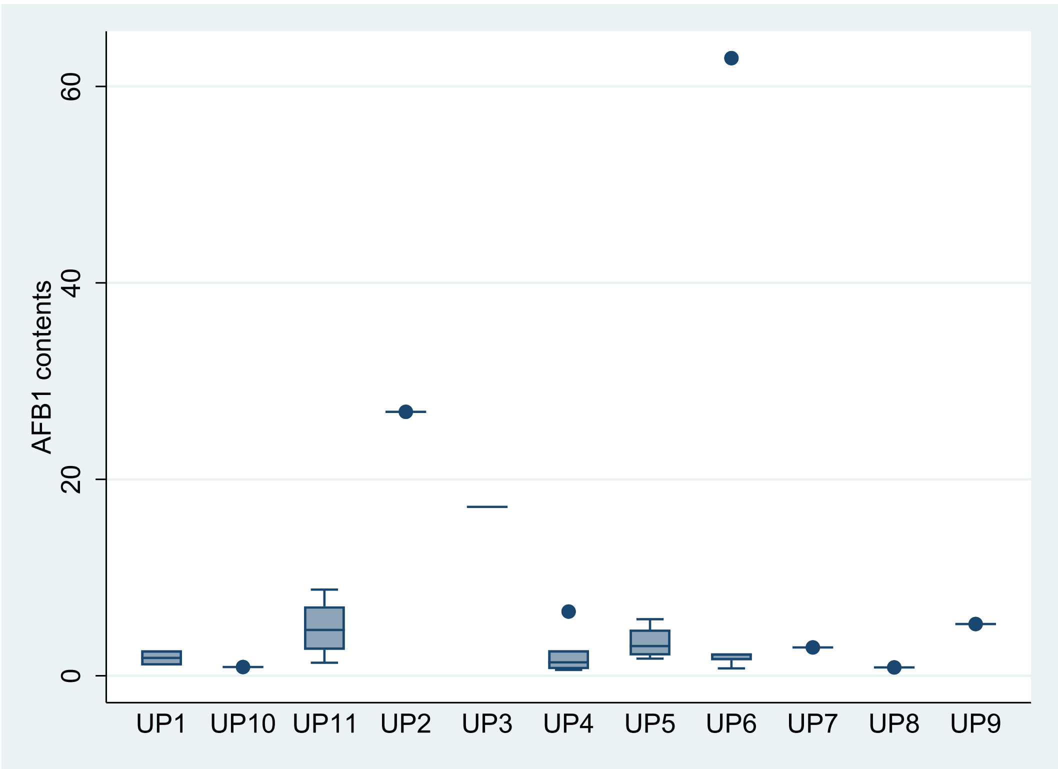

Figure 2. occurrence of AFB1 in infant flour according to the types of production units.

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4. Discussion

4.1. Occurrence of Mycotoxins in Raw Materials

This study revealed that all raw materials were contaminated with aflatoxins and Ochratoxin A. No sample of sorghum, maize, rice or cowpea was contained by fumonisin B1. Among these raw materials, the co-occurrence of the three types of mycotoxins was 7.7%. The results also showed that 77% of raw materials contaminated by total aflatoxins were out of the regulation limit of 4 µg/kg and that 80% among them had AFB1 levels above the limit of 2 µg/kg [18]. Raw materials samples had 30.8% of Ochratoxin A content above the commission regulation limit [18]. Previous studies have shown the effective presence of aflatoxins, fumonisins and Ochratoxin A in raw materials of West African countries [6,7,10,12,13,19] demonstrating the widespread contamination of these toxins in foodstuffs. In general, the variability of AFs, FB1 and OTA around the mean was high within the same raw material samples as well as between different types of raw materials. This variability could be explained by the origins of suppliers and the differences between the methods of conservation of the raw materials used by the infant flour production units. Indeed, raw materials suppliers are coming from different regions of Burkina Faso and West Africa with differences in rainfall, climate and storage practices. Nikiema *et al*., [10] have mentioned in their study that maize samples imported from the sub region contained higher mean level of fumonisins even relatively small number of samples from outside Burkina Faso should be noticed. Furthermore, a survey was conducted within the production units through an interview on the raw materials used in the production of infant flour and the use of storage pesticides. we noticed through this survey that some of them used storage pesticides (66%) while others (34%) did not [20]. In addition, some production units, unlike others, had stores equipped with storage straws. Storage conditions strongly influence the levels of mycotoxins in cereals and legumes [21]. The highest levels of total aflatoxins were found in peanuts and maize. According to Dieme *et al*., maize, peanuts, and other tree nuts are potential sources of exposure to aflatoxins [22]. Aflatoxins are produced in particular by A. flavus and A. parasiticus under some environmental conditions of high temperature and humidity frequently associated with tropical and subtropical climates [23]. In most West African countries, almost half of the cereal production has an aflatoxin content higher than international standards [22]. According to the commission regulation (EU) 2023/915 of 25 April 2023 relating to the maximum level for certain contaminants in foodstuffs, the maximum level of total aflatoxins is 4 µg/kg in cereals and cereal-derived products [18]. Among the aflatoxins, AFB1 is recognized as being the most toxic [1] and its maximum value in foodstuffs is set at 2 µg/kg [18]. Contamination of maize with fumonisins is common worldwide. Previous studies indicated the presence of fumonisin B1 in maize in Burkina Faso [24]. It is in fact the most frequently encountered mycotoxin in maize. However, the present study has found that soybeans, millet and peanut were the most contaminated raw materials intended for infant flours' production. On the contrary, fumonisin B1 was not detected in the different maize samples collected in our study. This could be due to the fact that most studies on fumonisin contamination of foodstuffs have been focused on maize than other speculations, hiding therefore the prevalence of this mycotoxin in other crops. Other explanations are the agricultural practices used, or by the fact that the conditions were unfavourable for fungal colonization of fumonisin B1-producing species. It should be noticed also that fumonisins are regarded as a field problem and that the differences in contamination may simply reflect annual variation in fungal colonization [25]. Indeed, studies have shown low levels of fumonisins B1 in corn samples taken directly from the field compared to samples stored for over a year [10]. This could be justified by the preventive measures taken throughout production since contamination can occur at all stages of the food chain. The maximum content of fumonisins (B1 and B2) in maize-based foods intended for consumption by infants set by the commission regulation (EU) 2023/915 of 25 April 2023 is 200 µg/kg. Fumonisin B1 is linked to oesophageal cancer in humans [1]. Furthermore, the consumption of food contaminated by fumonisins has been associated with a series of births of children with neural tube defects in the United States [26]. FB1 has immunotoxic effects and impairs both cytokine synthesis, the humoral-mediated immune response and the cell-mediated immune response and increases susceptibility to infections [27]. Fumonisins cause liver and kidney toxicity in animals. They also cause leukoencephalomalacia in horses and pulmonary edema.in pigs. Fumonisin B1 is carcinogenic in rats and mice [9]. The high content of Ochratoxin A in the soybean samples of this study could be explained by contaminations during storage. OTA is generally produced during the storage of raw materials in poor conditions [28]. Studies have revealed that OTA is frequently present in maize, millet, sorghum, peanut, sesame and soybean [28]. Among the different types of Ochratoxin, OTA is recognized as the most toxic, the most frequent and the best known [23]. The European Commission sets the limit value for Ochratoxin A in foodstuffs intended for human consumption at 3 µg/kg [18]. It is stable in storage and resistant to industrial transformation processes [28]. OTA was classified as possibly carcinogenic to humans by the IARC in 1993 [23]. Indeed, OTA has hepatotoxic, immunotoxic, genotoxic, teratogenic and carcinogenic effects. To combat the presence of mycotoxins (aflatoxins, fumonisins B1, Ochratoxins A) in raw materials, chemical, physical and biological methods, although limited, are used [22]. Studies have revealed antifungal properties of plants in inhibiting Arpergillus strains [29]. In addition, studies on raw material conservation practices are being carried out in Burkina Faso to identify the conservation techniques used by the population to improve their safety in terms of mould invasion [30].

4.2. Occurrence of Mycotoxins in Infant Flours

Aflatoxins, Fumonisins and Ochratoxin A are mycotoxins found in infant flours [27]. Most of the infant flours produced were contaminated by AFs, FB_1 and OTA with a very high variability between samples with a co-occurrence in 42.3% of the samples for the three mycotoxins. According to Commission Regulation (EU) 2023/915 of 25 April 2023 relating to maximum levels for certain contaminants in foodstuffs, more specifically concerning cereal-based foods intended for infants and young children, the Maximum Value in AFB1 is 0.10 µg/kg, 200 µg/kg for FB1, and 5 µg/kg for OTA. All infant flours were contaminated by aflatoxins with 96% above the regulation limit. Sixty-five point four (65.4%) of the infant formulas were contaminated by OTA, 68.8% of them being above the regulation limit while 57% were contaminated by FB1 but none of them being out of the regulation limit. In Burkina Faso, complementary foods are mostly prepared from nationally produced foods, such as cereals, oilseeds, legumes [31]. From our study, it appears that the distribution of mycotoxins (AFs, FB1 and OTA) in infant flours were highly variable within the same infant flour production unit and between the different types of infant flour production units. The high levels of AFB1 in infant flours produced in artisanal production units compared to those produced in semi-industrial units could be explained by the origin and the quality in the management of contaminants during the production processes. Studies have revealed that the quality of an infant flour is not linked to the type of unit that produces it, but to the training and monitoring of staff in compliance with good practices [32]. This was observed during surveys of these production units where it was established that many semi-industrial units would have better trained staff with much more rigorous monitoring compared to artisanal production units. The origin of the raw materials could also explain this high level of contamination in the infant flours produced by the artisanal production units. Most semi-industrial production units obtain their raw materials from their approved suppliers, while artisanal production units obtain their raw materials directly from markets from several traders. This supply chain increases the sources of contamination of raw materials [33]. When considering the results obtained on the occurrence of AFs, FB1 and OTA in infant flours, it appears that most of these infant flours produced are contaminated by AFs, FB1 and OTA with a very high variability between the contents. Depending on the doses, these mycotoxins can in the long term affect the health of infants and young children who consume infant flours. To do this, Tolerable Daily Intakes (TDI) have been determined in order to regulate their consumption. Concerning aflatoxin, given that it is the most genotoxic, immunosuppressive and carcinogenic mycotoxin, there is theoretically a no sub toxic dose. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommends risk management based on the ALARA (As Low as Reasonably Achievable) model. Thus, a quantity of 1 ng/kg of body weight per day has little risk of increasing the incidence of liver tumours in a population not affected by the hepatitis B virus [23]. The Tolerated Daily Intakes of fumonisins B1, B2, B3, separately or in

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combination is 2 µg.kg-1 bw per day [23]. With regard to OTA which accumulates in the body, the tolerable dose is in fact expressed as a weekly dose, i.e. 100 ng/kg bw/week [23]. Therefore, based on the carcinogenic effect of OTA, the TDI is also 5 ng.kg-1 bw per day.

5. Conclusions

At the end of this study, it appears that the levels of AFB1 in raw materials and in in-fant flours produced in artisanal production units were higher compared to those pro-duced in semi-industrial units. Given the presence of this mycotoxin in infant flours pro-duced locally and their effect on the health of infants and young children who are the main consumers, it is necessary that the various production units apply good hygiene and production practices. This would considerably reduce contamination during production and therefore guarantee a better sanitary quality of infant flours. Further studies should be conducted in a way to minimize contamination of mycotoxins in raw materials intended for infant flours production by using low cost chemical processes and sorting practices and the assessment of exposure to mycotoxins at individual level in infants and young children who are vulnerable groups in order to evaluate risk characterisation.

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References

1. Tozlovanu, M. Évaluation Du Risque de Contamination Alimentaire En Mycotoxines Néphrotoxiques et Cancérogènes (Notamment l'ochratoxine A): Validation de Biomarqueurs d'exposition et d'effet. PhD Thesis, 2008.
2. Moretti, A.; Logrieco, A.F.; Susca, A. Mycotoxins: An Underhand Food Problem. *Mycotoxigenic Fungi Methods Protoc.* **2017**, 3–12.
3. Huybrechts, B.; Tangni, E.K.; Debongnie, P.; Geys, J.; Callebaut, A. Méthodes Analytiques de Détermination Des Mycotoxines Dans Les Produits Agricoles: Une Revue. *Cah. Agric.* **2013**, *22*, 202–215.
4. JARD, G. Etude de Différents Modes d'élimination Biologique de La Zéaralénone, Mycotoxine Présente Dans Les Céréales: Adsorption et Biotransformation, Université de Toulouse: Toulouse, 2009.
5. Pleadin, J.; Frece, J.; Markov, K. Mycotoxins in Food and Feed. *Adv. Food Nutr. Res.* **2019**, *89*, 297–345.
6. Nikiema, P.A. Etude des aflatoxines au Burkina Faso. Détermination quantitative et qualitative des aflatoxines de l'arachide par des tests biochimiques et immunologiques, Univresité de Ouagadougou: Ouagadougou, 1993.
7. Ouattara-Sourabie, P.B.; Nikiema, P.A.; Traore, A.S. Caractérisation de Souches d'Aspergillus Spp Isolées Des Graines d'arachides Cultivées Au Burkina Faso, Afrique de l'Ouest. *Int. J. Biol. Chem. Sci.* **2011**, *5*.
8. Bationo, J.F.; Nikiéma, P.A.; Koudougou, K.; Ouédraogo, M.; Bazié, S.R.; Sanou, E.; Barro, N. Assessment of Aflatoxin B1 and Ochratoxin A Levels in Sorghum Malts and Beer in Ouagadougou. *Afr. J. Food Sci.* **2015**, *9*, 417–420.
9. Turner, P.C.; Nikiema, P.; Wild, C.P. Fumonisin Contamination of Food: Progress in Development of Biomarkers to Better Assess Human Health Risks. *Mutat. Res. Toxicol. Environ. Mutagen.* **1999**, *443*, 81–93.
10. Nikiema, P.N.; Worrillow, L.; Traore, A.S.; Wild, C.P.; Turner*, P.C. Fumonisin Contamination of Maize in Burkina Faso, West Africa. *Food Addit. Contam.* **2004**, *21*, 865–870.
11. Ware, L.Y.; Durand, N.; Nikiema, P.A.; Alter, P.; Fontana, A.; Montet, D.; Barro, N. Occurrence of Mycotoxins in Commercial Infant Formulas Locally Produced in Ouagadougou (Burkina Faso). *Food Control* **2017**, *73*, 518–523.
12. Warth, B.; Parich, A.; Atehnkeng, J.; Bandyopadhyay, R.; Schuhmacher, R.; Sulyok, M.; Krska, R. Quantitation of Mycotoxins in Food and Feed from Burkina Faso and Mozambique Using a Modern LC-MS/MS Multitoxin Method. *J. Agric. Food Chem.* **2012**, *60*, 9352–9363.
13. Afolabi, C.G.; Ezekiel, C.N.; Kehinde, I.A.; Olaolu, A.W.; Ogunsanya, O.M. Contamination of Groundnut in South-Western Nigeria by Aflatoxigenic Fungi and Aflatoxins in Relation to Processing. *J. Phytopathol.* **2015**, *163*, 279–286.
14. Ruppol, P.; Delfosse, P.; Hornick, J.-L. La Contamination de La Filière Laitière Par Les Mycotoxines: Un Risque Pour La Santé Publique En Afrique Subsaharienne. In Proceedings of the Annales de Médécine Vétérinaire; 2004; Vol. 2, pp. 141–146.
15. Shabeer, S.; Asad, S.; Jamal, A.; Ali, A. Aflatoxin Contamination, Its Impact and Management Strategies: An Updated Review. *Toxins* **2022**, *14*, 307.
16. Pei, X.; Zhang, W.; Jiang, H.; Liu, D.; Liu, X.; Li, L.; Li, C.; Xiao, X.; Tang, S.; Li, D. Food-Origin Mycotoxin-Induced Neurotoxicity: Intend to Break the Rules of Neuroglia Cells. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 1–14.
17. Amirahmadi, M.; Shoeibi, S.; Rastegar, H.; Elmi, M.; Mousavi Khaneghah, A. Simultaneous Analysis of Mycotoxins in Corn Flour Using LC/MS-MS Combined with a Modified QuEChERS Procedure. *Toxin Rev.* **2018**, *37*, 187–195.
18. Commission, E. Commission Regulation (EU) 2023/915 of 25 April 2023 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006. *J Eur Union* **2023**, *119*, 103–157.
19. Ezekiel, C.N.; Udom, I.E.; Frisvad, J.C.; Adetunji, M.C.; Houbraken, J.; Fapohunda, S.O.; Samson, R.A.; Atanda, O.O.; Agi-Otto, M.C.; Onashile, O.A. Assessment of Aflatoxigenic Aspergillus and Other Fungi in Millet and Sesame from Plateau State, Nigeria. *Mycology* **2014**, *5*, 16–22.
20. Bayala-Yaï, L.K.A.; Nikièma, P.A.; Ouédraogo, W.A.; Dembélé, I.; Nébié, Z.; Simpore, J. Management of Infant Flour Production in the City of Ouagadougou: A Survey Study. *PAMJ - One Health* **2024**, *14*, doi:10.11604/pamj-oh.2024.14.8.43373.
21. Bruce, A.C.C.; Kayode, A.P. Aptitude Au Stockage de Quelques Farines Infantiles à Base de Ressources Alimentaires Locales Du BENIN. **2017**, 90.
22. Dieme, E.; Fall, R.; Sarr, I.; Sarr, F.; Traore, D.; Seydi, M. Contamination Des Céréales Par l'aflatoxine En Afrique : Revue Des Méthodes de Lutte Existantes. *Int. J. Biol. Chem. Sci.* **2016**, *10*, 2285–2299, doi:10.4314/ijbcs.v10i5.27.
23. Brochard, G.; Le Bacle, C. Mycotoxines En Milieu de Travail. *Orig. Propr. Toxiques Princ. Mycotoxines INRS Doc. Pour Med. Trav.* **2009**, *119*.
24. Nikiéma, P.A. Etude des aflatoxines au Burkina Faso: Détermination quantitative et qualitative des aflatoxines de l'arachide par des tests biochimiques et immunologiques. **1993**.

 doi:10.20944/preprints202407.1840.v1

25. *Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene: This Publication Represents the Views and Expert Opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Which Met in Lyon, 12 - 19 February 2002*; International Agency for Research on Cancer, Ed.; IARC monographs on the evaluation of carcinogenic risks to humans; IARC: Lyon, 2002; ISBN 978-92-832-1282-9.
26. Hadjeba-Medjdoub, K. Risque de Multicontaminations En Mycotoxines et Moyens de Désactivation Par Les Parois de Levures et Levures Enrichies En Glutathion Ou Sélénométhionine. PhD Thesis, 2012.
27. GALTIER, P.; LOISEAU, N.; OSWALD, I.P.; PUEL, O. Toxicology of mycotoxins, hazards and risks in human and animal food Available online: https://doi.org/10.4267/2042/47807.
28. MAHNINE, N. Etude de La Contamination Des Produits Céréaliers Par Les Mycotoxines: Cas Des Aflatoxines, de l'ochratoxine A, Des Fumonisines et Des Mycotoxines Émergentes, Université Mohammed V: Rabat, 2017.
29. Ouattara–Sourabie, P.B.; Nikiema, P.A.; Traore, A. Antifungal Activity of Hyptis Spicigera (Lamiaceae) Extracts and Essential Oils of Cymbopogon Citratus (Poaceae) and Cymbopogon Giganteus against the Growth of Aspergillus Strains Isolated in Bur Ina Faso. *J Pharm Pharmacol* **2017**, *7*, 17–27.
30. Sankara, F.; Sanou, A.G.; Waongo, A.; Somda, M.; Toé, P.; Somda, I. Pratique Paysanne Post Récolte Du Maïs Dans La Région Des Hauts-Bassins Du Burkina Faso. *J. Anim. Plant Sci.* **2017**, *33*, 5274–5288.
31. Amoin, A.; Agbo, E.A.; Dago, A.G.; Gbogouri, A.G.; Brou, D.K.; Dago, G. Comparaison Des Caractéristiques Nutritionnelles et Rhéologiques Des Bouillies Infantiles Préparées Par Les Techniques de Germination et de Fermentation. *Int. J. Biol. Chem. Sci.* **2015**, *9*, 944–953.
32. Olive, F.; Mouquet-Rivier, C.; Fioroni, N.; Bichard, A.; Boulle-Martinaud, N.; Kaboré, C.; Denizeau, M.; Zagré, N.; Le Dain, A.; Ndiaye, N. La Filière Des Farines Infantiles Produites Localement Dans 6 Pays Sahéliens 2020.
33. Panisset, J.-C.; Dewailly, É.; Doucet-Leduc, H. Contamination Alimentaire. *Environ. Santé Publique Fond. Prat. Éditions TEC DOC Edisen* **2003**.