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Occurence of Mycotoxins in Traditional Dried Meat (Charmout) Sold in the Markets of Ndjamena, Chad

Brahim Adoum A.[1,2], Ali Haroun[1,2], Denis Erbi[3,2], Saturnin Naim[4], Al-Lamadine Mahamat, Abdoullahi Hissein O.[1], Abdelsalam Tidjani[1], Aly Savadogo[2,*]

[1]Laboratoire de Recherche en Sciences des Aliments et Nutrition (LARSAN), Faculté de Sciences de la Santé Humaine, Université de N'Djamena BP1117, Tchad
[2]Laboratoire de Biochimie et Immunologie Appliquées (LaBIA) Université Joseph Ki-Zerbo BP7021 Ouagadougou 03, Burkina Faso
[3]Université Adam Barka (Tchad)
[4]Centre de Contrôle de Qualité des Denrées Alimentaires (CCOQDA), BP4486 N'Djaména, Tchad
*Corresponding author: alysavadogo@gmail.com

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Abstract *Charmout* is a traditional product made from sun-dried meat, widely consumed in Chad. Its artisanal nature and exposure to the open air make it susceptible to contamination by various molds. The aim of this work was to evaluate the mycotoxins produced by molds in charmout. One hundred sixty-two samples were collected from the six (6) markets (central market, millet market, Al-afia market, Farcha market, Dembe market, and Al-adala market) in the city of N'Djamena and analyzed. The results showed that out of sixty-four contaminated samples, 41 were fungal flora, of which 39.50%; 26 were *Aspergillus niger*, 7 were of the *Mucor* genus, and 8 were of the *Fusarium* genus. The average concentration of aflatoxins ranged from 0.002 µg/kg to 0.04 µg/kg; from 0.004 µg/kg to 0.97 µg/kg; from 0.011 µg/kg to 0.35 µg/kg; and from 0.065 µg/kg to 0.075 µg/kg respectively for aflatoxins G2, G1, B2, and B1. For moisture content, the average values were 5.40±0.05; 9.78±0.53; 14.21±0.69; 13.28±0.84; 12.98±0.83; and 12.85±1.37, respectively in the samples from the central market, Millet market, Al-afia market, Dembe market, Farcha market, and Al-adala market. The samples from the Al-afia market and the Dembe market had moisture content levels higher than the value set by the Burkinabe Agency for Standardization, which is 13%.

Keywords: *charmout, aflatoxin, molds, N'Djamena, Chad*

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1. Introduction

The sanitary quality of food products can be threatened by all kinds of contaminants, particularly natural toxins. Among these are mycotoxins, which refer to the molecules produced by filamentous fungi (molds) and pose a risk to the health of humans and animals [1].

Mycotoxins are a major global concern for public health due to their various toxic effects. Additionally, they lead to enormous economic losses [2,3,4,5]. They are natural toxins produced by certain fungi that grow on various foodstuffs and animal feed under pre-harvest and storage conditions, and they are toxic to both humans and animals [6,7]. Mycotoxins are produced by potentially toxinogenic molds, primarily belonging to the genera *Fusarium*, *Aspergillus*, *Penicillium*, *Claviceps*, and others [8].

Among mycotoxins, the group of aflatoxins is the most well-known, the most studied, and the most regulated. However, three main strains of *Aspergillus* (A. *flavus*, A. *parasiticus*, and A. *nominus*) are known for their ability to naturally synthesize aflatoxins under warm and humid conditions. Aflatoxins pose a serious threat with potentially devastating effects on health, often going unnoticed in our everyday food [10]. AFB1 accounts for more than 75% of all aflatoxin contamination in food and animal feed [11].

To protect consumer health, some countries have set maximum levels (5.0-10.0) µg/kg for mycotoxins in food [12], with the strictest limits established by the European Commission Regulation 1881/2006 (5.0-10.0) µg/kg. The produced *charmout* is exposed to unsanitary conditions and is susceptible to contamination by mold that produces mycotoxins. Among the food items that are often contaminated by mycotoxins is poorly stored *charmout*, especially during the rainy season. One of the issues that affects their quality and food safety also concerns the development of microorganisms in food. Particularly aflatoxigenic fungi such as *Aspergillus flavus* and *Aspergillus parasiticus*. The presence of spores and these species of fungi in food remains a major challenge for storage and preservation. The exposure of a population to mycotoxins is estimated by combining the concentrations

of mycotoxins in food with dietary consumption data.

In developing countries like Chad, if all food resources are not stored under proper conditions, there is a risk of increasing the level of food insecurity among the population. In the same vein, [15] reported that the contamination of kundi by aflatoxin in Nigeria is due to inappropriate conditions. These mycotoxins are aflatoxins (AFs), which have been shown to be potentially carcinogenic…[16]. Similarly, some of these toxins are thought to be carcinogenic or mutagenic, while others are toxic to the kidneys, nervous system, or liver. Furthermore, it is important to note that toxicity does not necessarily come from the mycotoxin itself, but can be due to one of its metabolites resulting from its degradation. [17]. The most toxic mycotoxins are those produced by the mold *Aspergillus flavus*; known as aflatoxins B1, B2, G1, and G2, these substances are highly carcinogenic (causing liver tumors). The foods most affected are peanuts and corn, which are produced in tropical/subtropical regions where the temperature and humidity are conducive to the biosynthesis of aflatoxins. However, few studies have been conducted in Chad on the isolation of molds and the determination of aflatoxins in *charmout*. This highlights the importance of our work, which aimed to analyze the mycotoxins produced by molds in *charmout* sold in the markets of N'Djamena, Chad.

2. Materials and Methods

2.1. Sampling

A total of One Hundred Sixty-Two (162) samples were randomly collected from six markets in the city of N'Djamena (Central Market, Millet Market, Farcha Market, Al-Adala Market, Dembe Market, and Al-Afia Market) (Figure 1). Twenty-seven samples (50 grams per sample) were collected from each market and transported to the laboratory in an aseptic manner, then stored at 4 °C while awaiting analysis. Twenty-four (24) contaminated samples were selected for aflatoxin testing, with 4 samples from each market due to their fungal density (black colonies).

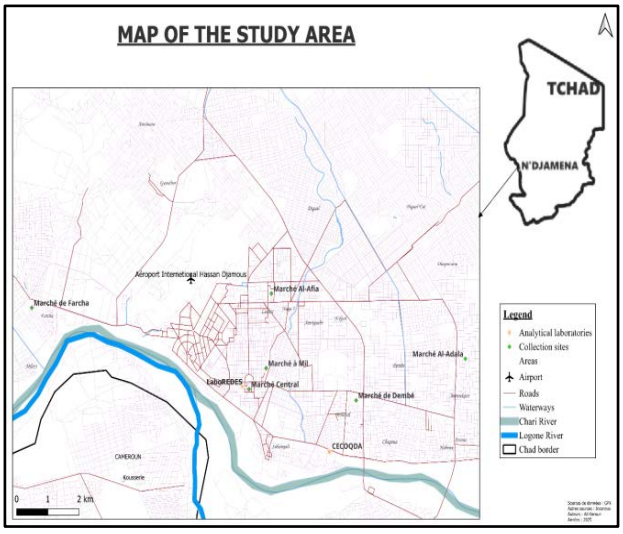


Figure 1. Sampling site and location of LaRSAN

The distribution of samples based on the quantity collected from various markets in the city of N'Djamena is presented in Table 1 below.

Table 1. Coding and distribution of samples collected from different markets

Coding	Sampling area	Quantity (g)	Number of samples collected
CHMM	Millet market	50	27
CHMD	Dembe market	50	27
CHMF	Farcha market	50	27
CHMAF	Al-afia market	50	27
CHMC	Central market	50	27
CHMAD	Al-Adala market	50	50

2.2. Culture and Isolation of Molds

Decimal dilution series were performed for each sample. The 10^{-2} and 10^{-3} dilutions were selected. Two Petri dishes containing *Sabouraud* agar with chloramphenicol were inoculated and incubated at 25 °C for 1 to 5 days. Colonies with characteristics of molds were isolated and preserved for further identification [18].

2.3. Morphological Identification and Cultural Aspects of Isolated Molds

The strains were inoculated onto *Sabouraud* medium with chloramphenicol (BIOLAB) for the macroscopic observation of colonies (size, color, shape, colony outline, etc.)

The morphological identification and cultural aspects of isolated molds were determined using a 5-day culture incubated at 25°C in chloramphenicol *Sabouraud* medium. The morphology and cultural aspects of the colonies (size, color, shape, colony contour, etc.) were obtained through macroscopic observation with the naked eye [19].

All the pure strains obtained were subjected to morphological identification through macroscopic and microscopic observation. The microscopic observation of the fungal cells was performed using a fragment of the pure strain taken with a sterile platinum loop. This fragment was placed between a slide and a coverslip, onto which lactophenol blue was added as a diluent. The microscopic observation was conducted at magnifications of x10 and x40 [20].

2.4. Determination of Moisture Content

The determination of water content was carried out according to the standard NBF01-087: 2009. 5 g of the ground *charmout* sample, using an electric grinder, were placed in crucibles that had been previously washed, dried

in an oven for 1 hour, and cooled in a desiccator for 30 minutes. The crucibles containing the samples were then placed in the oven at 105°C for 12 hours, and subsequently in the desiccator for 10 minutes. Next, the mass of the test sample. after drying was determined. Thus, the moisture content was calculated using the following formula:

$$\mathbf{H}(\%) = \frac{M1 - M2}{PE} \times 100$$

PE = test weight in grams

M1 = mass in grams of the container and the test weight

M2 = mass in grams of the container and the test weight after drying

2.5. Measurement of Aflatoxins B1, B2, G1, and G2 in Samples

2.5.1. Extraction

Twenty-five grams (25 g) of each sample were weighed and ground to obtain a fine powder that allows for the release of toxins. Then, 5 g of each obtained powder is introduced into a small plastic bottle containing 125 ml of the previously prepared extraction solution (70% methanol / 30% water). The flask is then shaken for 2 minutes at an average speed. A Wathman filter paper (qualitative grade 1V D.240MM-PAR 100) with a diameter of 24 cm and a porosity of 11 µm is used to filter the previous solution, then 15 ml of the filtrate is introduced into a conical flask, and 30 ml of water is added. The mixture is filtered again using a glass microfiber filter paper (11 cm in diameter) to ensure that the filtrate is clear. ISO 16050/2003.

2.5.2. Purification

Using a pipette, 15 ml of filtrate was placed in the immunoaffinity column (ref Brownlee Validated C18-USA), and the column was washed with water. The eluted fraction was collected by passing 0-5 ml and then 0-75 ml of pure methanol through the column, thus trapping the aflatoxins in a 5 ml vial and diluting them with water.

Mycotoxins were analyzed according to the ISO 16050/2003 method using ultra-high performance liquid chromatography (UHPLC) from Perkin Elmer.

2.5.3. Establishment of the Calibration Curve and Calculation of Aflatoxin Levels

The calibration curve was standardized using solutions with known concentrations. Each solution was injected into the chromatography, and the areas of the obtained HPLC peaks were recorded. A line was then drawn to represent the relationship between the concentrations of the standards and the corresponding areas. The equation of this line (Y=aX+b) was used to determine the concentration of aflatoxins present in the analyzed charmout samples. This curve is therefore designed to accurately quantify aflatoxins in a food product structure.

2.6. Statistical Analysis of Results

For statistical processing, the R software was used for data entry, calculation of means, and standard deviation. These data were subjected to an analysis of variance (ANOVA) using R software to determine statistical differences at a significance level of P = 0.05.

3. Results and Discussion

3.1. Contamination of Samples by Molds

Out of the 162 samples collected, 64 were contaminated with mold. 41 were isolated due to their fungal characteristics (colonies, size, shape, and contour) in order to identify them. The contamination rate is around 39.50%, which is significantly lower than the 91.4% found by [20] based on a total of 57 samples. Considering that fungi thrive in humid areas, this could be explained by the fact that the drying process of the *charmout* is inadequate.

The mold load in the samples from the Farcha Market consisted of eight (08) types. Four (04) were A. *niger*, two (02) were *Mucor*, and two were *Fusarium*. The samples from the Al-Afia market were also contaminated with mold, totaling 11 types (9 A. *niger*, 1 *Mucor*, and 1 *Fusarium*). The mold load in the samples from the Dembe Market was the highest, with 17 types of molds (10 A. *niger*, 3 *Mucor*, and 4 *Fusarium*). The samples from the *charmout* of the Al-adala Market contained 5 types of molds (3 A. *niger*, 1 *Mucor*, and 1 *Fusarium*). The samples from the Central Market and the Millet Market showed no mold load. This could be explained by adherence to Good Manufacturing Practices and Good Hygiene Practices.

Table 2. Distribution of isolated molds based on Markets

Sampling area	Isolated mould		
	A. niger	*Mucor sp*	*Fusarium sp*
Central market	0	0	0
Millet market	0	0	0
Al-afia market	9	1	1
Dembe market	10	3	4
Farcha market	4	2	2
Al-adala market	3	1	1
Total	26	7	8

3.2. Different Species of Molds Isolated from the Samples and Identified by Macroscopic and Microscopic Methods

The isolation carried out from the samples of different markets in Ndjamena (Table 1) resulted in the collection of 64 fungal strains. The microscopic characteristics of the molds are recorded in Table 3 below. The cultural characteristics of the isolated fungal strains (macroscopic) have also been summarized in the same table.

3.3. Frequency of Isolated Species

Figure 3 shows the frequency of isolated mold species. These results indicate that out of the one hundred sixty-two samples (162), 41 (64.06%) are *Aspergillus niger*, 10 (15.63%) are *Mucor sp*, and 13 (20.21%) are *Fusarium*. Meat is a food that is very rich in nutrients such as protein, iron, zinc, silicon, phosphorus, and vitamin B12 [21]. However, due to its high nutrient content, meat provides a favorable environment for the growth of spoilage

microorganisms and foodborne pathogens [22]. The presence of mold in the samples could be explained by environmental contamination due to non-compliance with good manufacturing practices. For example, it is noted that women handle the meat with their bare hands in the open air (Figure 2).

Fungal contamination of food products can lead to a decrease in their nutritional value as well as the deterioration of their organoleptic qualities. If the strains are toxin-producing molds and if environmental conditions are favorable, there can be synthesis and accumulation of toxins [23].

Table 3. Characteristics of Fungal Strains - Microscopic and Macroscopic Aspects

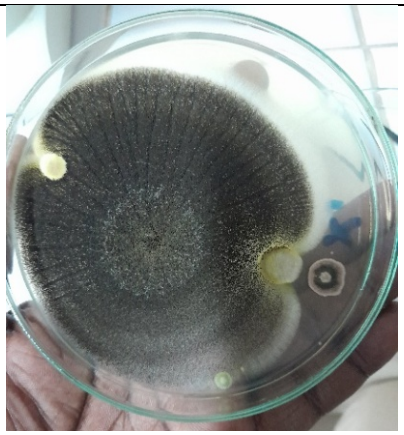
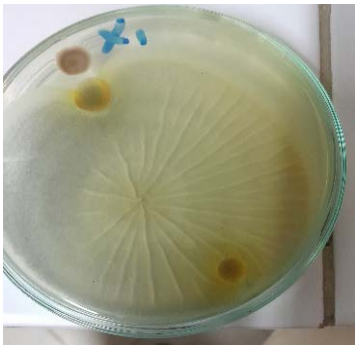
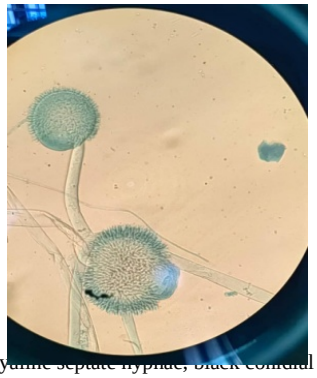
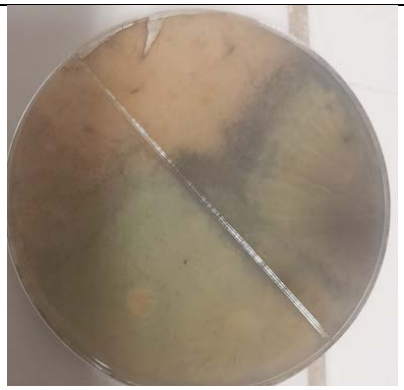
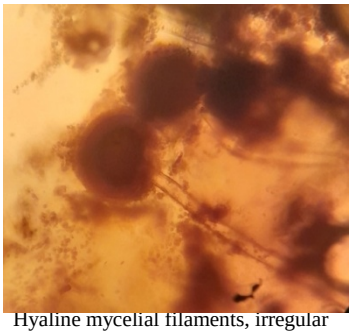
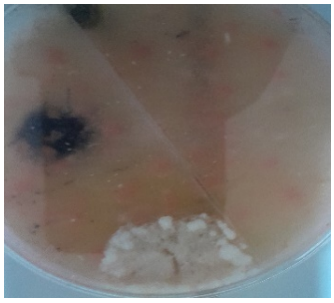
Fungal species	Macroscopic aspect		Microscopic aspect x 40
	Recto	Verso	
Aspergillus niger *A. niger*: blackish, powdery colonies on the front. The reverse side is gray, with rapid growth.			 Hyaline septate hyphae, black conidial heads and globose conidia. Irregular ridges
Mucor sp *Mucor*: gray woolly appearance on front and back, beige or white color			 Hyaline mycelial filaments, irregular contours, thin walls with little or no septation.
Fusarium sp *Fusarium*: flat colonies on the front and woolly to cottony whitish colonies on the back.			 They are transparent, *fusiform*, often multicellular, curved and thin-walled.

Figure 2. A woman pouring *charmout* onto a bag

3.4. Water Content of the *charmout*

The results of the moisture content found in the *charmout* samples are recorded in Table 4 below.

Table 4. Moisture content of charmout in the six (6) markets of the city of N'Djamena.

Codes	Humidity %	Average
CHMC	5,66±0,01	
CHMC	4,84±0,12	
CHMC	5,49±0,04	5,40±0,05
CHMC	5,60±0,00	
CHMM	5,71±0,01	
CHMM	5,33±0,16	
CHMM	14,13±0,69	9,78±0,53
CHMM	13,96±1,17	
CHMAF	13,60±1,64	
CHMAF	14,62±1,07	
CHMAF	13,97±0,07	14,21±0,69
CHMAF	14,65±0,48	
CHMD	13,58±1,28	
CHMD	13,40±0,40	
CHMD	14,33±0,62	13,28±0,84
CHMD	11,82±2,27	
CHMF	13,17±0,40	
CHMF	13,90±0,16	
CHMF	13,22±0,62	12,98±0,83
CHMF	11,65±2,00	
CHMAD	14,42±0,83	
CHMAD	12,00±3,39	
CHMAD	11,18±1,34	12,85±1,37
CHMAD	13,80±0,22	

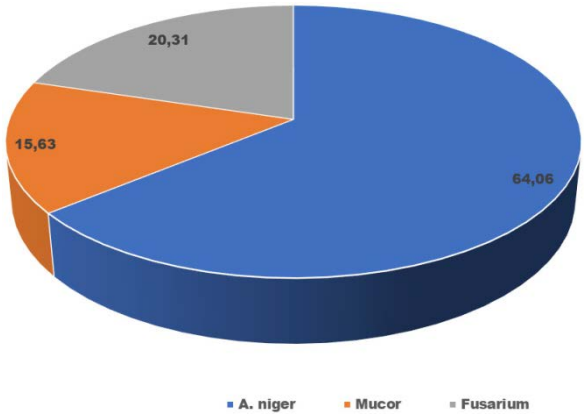


Figure 3. Percentage of isolated molds

3.5. Aflatoxins Present in *charmout*

Figure 4 shows that the found values of aflatoxin G2 range from 0.002 µg/kg (E9) to 0.04 µg/kg (E24), while those for aflatoxin G1 range from 0.004 µg/kg (E16) to 0.97 µg/kg (E14). As for the values of aflatoxin B2, they range from 0.0011 µg/kg (E24) to 0.35 µg/kg (E14), and from 0.065 µg/kg (E14) to 0.075 µg/kg (E15) for aflatoxin B1. Aflatoxin G1 was found more frequently in the samples than the other aflatoxins (G2, B2, and B1). The high presence of aflatoxins in the charmout samples from certain markets such as Dembe and the Al-afia market could be explained by the high moisture content in these samples. Indeed, these samples showed moisture levels exceeding the limit set by the Burkinabe Agency for Standardization, which establishes a maximum value of 13%.

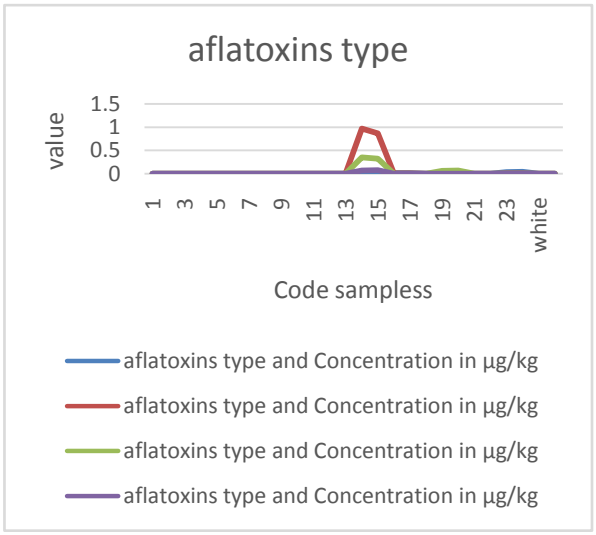


Figure 4. Types of aflatoxins and concentration in µg/kg

Result of the ANOVA statistical test of microbiological parameters based on locations

Overall case of all samples

The result allows us to test the significant differences between the means of the different types of aflatoxins and the total concentration of samples collected from the *charmout* in the 6 markets of N'Djamena.

Table 6 presents the results obtained from the aflatoxin assay in the charmout.

The results of the mycotoxin analyses produced by the *charmout* compared to the threshold set by European Regulation 1881/2006 (5.0-10.0) µg/kg are illustrated below:

For all the samples, the result allows us to illustrate the overall correlation of the charmout samples collected from the 6 markets in N'Djamena.

Table 5. Concentration of different types of aflatoxins (µg/kg) from various markets

	Aflatoxins type				Total concentration in µg/kg
	G2	**G1**	**B2**	**B1**	
Central market	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0000±0,0000
Millet market	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0000±0,0000
Al-Afia market	0,0011±0,0012[a]	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0000±0,0000[a]	0,0032±0,0036
Dembe market	0,0053±0,0045[a]	0,4610±0,5316[a]	0,1675±0,1938[a]	0,0350±0,0406[a]	2,0065±2,2956
Farcha market	0,0038±0,0075[a]	0,0011±0,0022[a]	0,0333±0,0387[a]	0,0000±0,0000[a]	0,1143±0,1021
Al-Adala market	0,0189±0,0219[a]	0,0033±0,0039[a]	0,0008±0,0010[a]	0,0000±0,0000[a]	0,0690±0,0799
Overall average	0,0048±0,0109[a]	0,0776±0,2599[a]	0,0336±0,0948[a]	0,0058±0,0198[a]	0,3655±1,1196
P-value	0,0947	0,0385 *	0,0512	0,0398 *	0,041 *

Table 6. Measurement of Different Types of Aflatoxins

Aflatoxins type	Number	Percentage
G2		
Positif	08	33,3
Negatif	16	66,7
Total	24	100
G1		
Positif	06	25,0
Negatif	18	75,0
Total	24	100
B2		
Positif	06	25,0
Negatif	18	75,0
Total	24	100
B1		
Positif	02	8,3
Negatif	22	91,7
Total	24	100
CT (µg/kg)		
Positif	10	41,7
Negatif	14	58,3
Total	24	100

Table 7. Assessment results of compliance levels for samples of different types of aflatoxin based on markets

Market	Appreciation	Aflatoxins type and Concentration in µg/ml				CT (µg/kg)
		G2	G1	B2	B1	
Central	*S	-	-	-	-	-
Millet	*S	-	-	-	-	-
Al-afia	*S	-	0,2-2,0	-	-	0,006-0,0006
Dembe	*S	-	5,06-10,0	-	-	0,005-4,17
Farcha	*S	-	0,0-10,0	-	-	0,18-0,21
Al-adala	*S	-	0,0-3,0	-	-	0,12-0,14
Total	S					
	US					

*S: Satisfactory ; **US: Unsatisfactory ; * limite Accepted by le regulation (UE) N° 1881/2006 (5,0-10 µg/kg)

Table 8. Test results for correlation between different types of aflatoxins based on markets

		G2	G1	B2	B1	CT (µg/kg)
G2	R	1	0,017	-0,014	0,007	0,038
	P		0,938	0,948	0,974	0,862
G1	R	0,017	1	0,980**	0,991**	0,998**
	P	0,938		0,000	0,000	0,000
B2	R	-0,014	0,980**	1	0,973**	0,988**
	P	0,948	0,000		0,000	0,000
B1	R	0,007	0,991**	0,973**	1	0,991**
	P	0,974	0,000	0,000		0,000
CT (µg/kg)	R	0,038	0,998**	0,988**	0,991**	1
	P	0,862	0,000	0,000	0,000	

The total aflatoxin dosage performed by HPLC on the *charmout* revealed that the results of our samples were below the maximum allowable residual content (5.0-10.0 µg/kg) according to European regulation 1881/2006. The results obtained by Bulent Kabac (2021) [23] were higher than ours. The average concentrations found in our *charmout* samples were higher than those found by Tina *et al.* (2022) [24] in dried meat. Furthermore, the average concentration of B1 (5.9±1.9) obtained in the *charmout* samples was lower than those found by Fahim *et al.* (2014) [25] (13.38±1.52) in *Kofta* meat and also lower than those reported by Ismail *et al.* (2013) (10.4±5.1) in Luncheon meat. The presence of aflatoxins in meat poses significant health risks to consumers and has led to numerous publications (Houshman *et al.*, 2024) [26].

The total concentration in the *charmout* samples found to be 26.66% does not corroborate with that obtained by Houshman *et al.* (2024) [26] in the MOP samples (meat products and offal).

The samples from the Central market, Millet market, Farcha market, and Al-adala market have a moisture content lower than the standard set by ABNORM (13%). Meanwhile, the samples from the Dembe market and Al-afia market have a moisture content higher than the standard set by ABNORM (Table 4). Furthermore, the moisture content obtained by Ali Haroun *et al.* (2023) [27] in the *charmout* was higher than our results. However, the difference in moisture content of the *charmout* is significant across the various markets in the city of N'Djamena. These discrepancies could be explained by a differential humidity of the *charmout* during drying or the way it is spread on the ground in certain markets, as shown in figure 2. Ali. H [27] reported higher values (14.33±2.82) for the *charmout*; this difference could be explained by the fact that his samples were collected from peripheral markets in different provinces, which do not have production units as suitable as those in N'djamena.

Statistically, the difference associated with the examined *charmout* was not significant ($P < 0.05$) among the different markets regarding the average concentration of G2 and B2. However, the average concentration of B1 and G1 is statistically significant (0.0398 and 0.0385) $P < 0.05$.

4. Conclusion

The results indicate that the contamination of *charmout* by mycotoxins in the markets of N'Djamena is satisfactory, despite the high humidity levels in some markets. Furthermore, the majority of the isolated fungal flora was *Aspergillus*, which has the ability to secrete aflatoxin. This allowed us to measure the aflatoxins, and the levels found are not too high, in accordance with European Regulation 1881/2006, which sets a limit of 5.0-10.0 µg/kg. The levels of aflatoxins B1 and B2 varied significantly between samples from different markets. The presence of mold in the *charmout* samples could be due to poor drying or storage conditions. This study highlights the need to raise awareness among producers and sellers of *charmout* in the various markets of N'Djamena, Chad, about good manufacturing practices and good hygiene practices. It confirms that the growth of toxic molds does not necessarily imply the presence of mycotoxins in food, as their production depends on a number of environmental factors [28].

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