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Aflatoxin and Fumonisin in Corn Production Chain in Bafia, Centre Cameroon: Impact of Processing Techniques

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Abstract: Food safety is to be a vital component of food security, with mycotoxin contamination, a major contributing factor. In line with this, this study aimed at investigating the effect of maize maturity at harvest, and processing techniques on the aflatoxin and fumonisin levels in maize and maize products. Three maize maturity stages (80, 85, and 90 days after sowing), two drying processes (sun and barn drying), three storage periods (one, two and three months) and subsequent maize derivatives under these conditions were sampled. These were analysed for total aflatoxins and total fumonisins using quantitative ELISA and samples with total aflatoxins and total fumonisins exceeding regulated levels were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine the sub-types of toxins present. Results obtained showed that all analyzed samples were contaminated with total aflatoxins (range: 0.8 to 20 μg/kg) and total fumonisins (range: 10 to 5990 μg/kg). Sun or barn drying for one week followed by one month usual storage resulted in significant total fumonisins contamination, emphasizing the need of at least two weeks of drying maize. It was also observed that processing techniques partly reduced the levels of toxins, mainly in maize products that have a sieving step.

Key words: Maize, processing, total aflatoxins, total fumonisins, Cameroon.

1. Introduction

Mycotoxins are thermally stable toxic secondary metabolites of molds that are ubiquitous in nature. There are more than 300 mycotoxins known to exist in nature but those most commonly encountered in the tropics are the aflatoxins and fumonisins [1]. Both AFs and FBs commonly contaminate maize and its products. In Cameroon where environmental conditions particularly favor the proliferation of toxigenic molds in foods, maize-based dishes are the staple food of about 12 million people and thus, we can infer that a majority of the population are probably exposed to mycotoxins by dietary means [2]. Within the last two decades in the country, mycotoxins have been the subject of further research. A positive correlation between storage duration and fumonisin B_1 (FB_1) levels in maize samples from the rainforest and the highland regions of Cameroon was previously established by [3]. Njobeh et al. [4] showed the simultaneous occurrence of mycotoxins in foods marketed in selected regions in this country, while it has been demonstrated that human exposure to aflatoxins is a potential risk factor to increase incidence of malnutrition and cancer [5]. Furthermore,

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Nguegwouo et al. [6] showed that agricultural practices in Bafia constitute risk factors for contamination of maize by fumonisins, Abia et al. [7] showed multi-mycotoxin contamination in some commonly consumed foods and Njumbe et al. [8] reported high levels of fumonisins, AFB_1, roquefortine C, and DON (deoxynivalenol) in maize from three agro-ecological zones. In continuation of these earlier studies, the objective of our work was to investigate the effects of maize maturity stage at harvest and processing techniques on the level of total aflatoxins and total fumonisins in maize and maize products in a targeted Cameroonian pole of high maize production and consumption.

2. Experimental Section

2.1 Standards, Reagents and Instruments

All reagents used in this study were of analytical grade, except where otherwise stated. Commercial ELISA kits were obtained from Renekabio, (USA) while an ELISA plate reader (Biotek, EL x 800, USA) was used to obtain the optical density. The materials supplied were antibody coated microwell, dilution well (green), aflatoxin standards (0.0; 1.0; 2.5; 5.0; 10.0; 20.0 µg/kg), fumonisin standards (0.0; 10; 100; 800; 2000; 6000 µg/kg), aflatoxin horseradish peroxidase (HRP)-conjugate, fumonisin HRP conjugate, substrate reagent (tetramethylbenzidine or TMB); stop solution (acidic solution) and BST wash buffer powder. The LC-MS/MS used was made up of Micromass QuattroLC triple quadrupole mass spectrometer, Water Alliance LC system2695, Edwards rotary pumpE2M28 Compaq Professional Workstation AP200, Monitor COMPAQ, HP Laser2420,column (Symmetry C_{18}, 5 µm150 x 2.1 mm, WAT056.975HK6, Waters) and guard column (Symmetry C_{18},3.5µm10 x2.1 mm, WAT106127HK7, Waters). Standards used for analysis were aflatoxin B_1, B_2 (Oskar Tropitzsch, Marktredwitz, Germany), fumonisin B_1, B_2 (Oskar Tropitzsch, Marktredwitz, Germany) and internal standard zearalanone (Sigma Aldrich, Bornem, Belgium).

2.2 Sample Collection and Preparation

The study was carried out between March and December 2012 in *Bafia* (4°45′N 11°13′E) located 120 km North of *Yaoundé* in the agro-ecological humid zone of Cameroon. The following samples were collected from three randomly selected villages (*Donenkeng I, Binya*, and *Tchekané*); primary maize samples including: 1 kg each of yellow fresh maize grains harvested at 80 (less mature), 85 (semi mature) and 90 (mature) days from sowing respectively; sun dried mature maize for one and two weeks and storage for two weeks; barn dried mature maize for one and two weeks and storage for two weeks; storage mature fresh yellow maize for one, two and three months. In the same way, primary sample of major ingredient of maize products and dishes included 1 kg of each collected sample in three selected villages (*Donenkeng I, Binya,* and *Tchekané*). The dishes included maize beer; dry or fresh maize cake with vegetables; dry or fresh maize cake with groundnut; maize porridge; maize *fufu* (*couscous*) which have sorting and sieving phases, maize milk; vegetables with maize; roasted maize; boiled maize; fried maize with groundnuts. The maize products were processed using the techniques described by Nguegwouo et al. [6] with slight modifications (Table 1). In our research, 104 primary maize samples and products were collected from three villages then mixed to obtain 38 composite samples from which 500 g was extracted finally. Solid samples were dried at 50 °C for four days and then reduced to powder by milling, while liquid samples (maize porridge and maize beer) were lyophilized and reduced to powder in the same manner. Total aflatoxins and total fumonisins levels were assessed on all pulverized composite samples while AFB_1, AFB_2, FB_1 and FB_2 were evaluated by LC-MS/MS (high performance liquid chromatography coupled to mass spectrometry) on some selected composite samples. Three samples were analyzed per

Table 1 Products derived from maize and their local processing methods (modified from [6]).

Maize-based dishes	Major ingredients (minor ingredients)	Local processing methods
Maize vegetables (Safon* or sanga*)	-2.5 kg of immature maize, (300 g of vegetable [Solanum nigrum], 300g kwem [Manihot esculenta leaves], 300 g of palm nuts)	Cut up immature maize grains Chop vegetable Cook the mixture of the two items Add the extracted oil and cook for one hour
Fresh or dry flat maize cake with vegetables (kpwindim*)	3 kg of dry or fresh maize (300 g of vegetable, 10 g of salt, 100 g of palm oil)	Sort good grains and grind Sieve the flour and add the water Wrap it in leaves and cook with vegetable for one hour Pound with the vegetable in a mortar and add palm oil
Fresh or dry flat maize cake with groundnuts (Kekumba*)	3 kg of dry or fresh maize, (300 g of groundnuts, 10 g of salt and 10 g of sugar, 100 g of palm oil)	Sort good grains and grind Sieve the flour and mix the grind groundnuts Add palm oil, salt and a bit of sugar Wrap it in banana leaves and cook for one hour
Maize porridge (Kenouk*)	3 kg of dry or fresh maize (200 g of sugar)	Sort good grains and soak for three days Grind and sieve Allow the pap to ferment for a day Put diluted pap into this boiled water while stirring Add water and sugar as to taste
Maize fufu (Couscous) (Kepen*)	3 kg of dry maize	Sort good grains and grind them Sieve and wash the chaffs Put the wash bran in boiled water and cook for 20 minutes Add the sieved flour and mix Add water progressively till the paste is well soft
Maize milk (Melek me Baazi*)	2 kg of dry maize	Sort good grains Grind dry maize Sieve the ground maize Grill the sieved flour for one hour
Maize beer (Kwata*)	5 kg of dry maize	Sort good grains Grind dry maize Soak the dry maize in water Drain using a sieve and dry under the sun Soak some other grains and allow to ferment Ground these fermented grains on the stone Mix both the sun-dried and fermented maize flours and grind Cook for more than an hour After cooking, allow it to ferment in the pot for three days
Roasted maize (Baazihangue*)	2 kg of dry maize	Sort good grains Put them on fire and grill for 30 minutes
Boiled maize(KeBaazi*)	2 kg of fresh maize	Remove the shucks of fresh maize Boil for one hour
Maize fritters (Kpwaa*)	2 kg of dry maize (200 g of ripe banana, 100 g of sugar, 200 g of palm oil, 10 g of salt and 10 g of yeast)	Sort good grains and sieve the ground Add ripe banana and stir to obtain a homogenous paste Add sugar, yeast and mix Fry in hot palm oil for about 10 minutes
Fried maize with groundnuts (Baazihangue de keezoo*)	2 kg of dry maize (300 g of groundnuts)	Sort good grains Grill grains with groundnuts for 30 minutes

* Vernacular names in Bafia (centre-Cameroon).

step of treatment (with an ELISA kit) and the average taken. However, for LC MS/MS only composite samples that exceeded the recommended level with ELISA Test were re-analyzed. The levels of toxins were expressed in μg/kg of dried maize or product content.

2.3 Immunoenzymatic Analysis of Total Aflatoxins and Total Fumonisins Using Quantitative ELISA

Total aflatoxins and total fumonisins presented in composites samples of maize and maize-products were analyzed using a competitive ELISA according

to the manufacturer's instruction (RENEKABIO, No: AF012714, No: FU012714, USA). Briefly, total fumonisins were extracted from 20 g of a ground sample with 40 mL of 90% methanol. After introducing 100 µL of conjugate solution A (green) into the appropriate dilution wells followed by 100 µL of conjugate B (clear); 100 µL of each standard or extracted sample were added. The content of each dilution well was mixed 3 times and 100 µL were transferred to a corresponding antibody coated microtitter well and incubated at room temperature for 20 minutes. The microwells were decanted and washed with 100 µL of PBS tween wash buffer 3 times. The substrate reagent (100 µL) was added to each microwell followed by incubation after covering to avoid direct light for 10 minutes and the blue color was developed. The acidic stop solution (100 µL) was added in each microwell which changed color from blue to yellow.

The optical density (OD) from each microwell was recorded with a microtiter plate using a 450 nm filter. The intensity of the color was inversely proportional to the concentration of fumonisins in the sample or standard. The optical densities of the samples were compared to the optical densities of the kit standards and an interpretative result was determined. Total aflatoxins were extracted from 20 g of a ground sample with 100 mL of 70% methanol. After introducing 200 µL of the conjugate into each dilution well, 100 µL of each standard or extracted sample were added. The following procedure was the same with the total fumonisins but after decanting, each dilution well was washed using 100 µL of distilled or desionized water 5 times. The recovery percentage was determined using reference material (FAPAS TEST MATERIAL SPECIFICATION SHEET, TO4138) from manufacturer and found to be higher than 90% in all cases. Limit of detection (LOD) and limit of quantification (LOQ) for total aflatoxins and total fumonisins were < 0.01 and 0.01 µg/kg; < 10 and 10 µg/kg respectively.

2.4 LC-MS/MS Conditions

AFB_1, AFB_2, FB_1 and FB_2 in selected maize and processed samples were analyzed using an LC-MS/MS method as described by Njumbe et al. [8]. Briefly, the detection and quantification were performed with a Waters UPLC mass spectrometry Micromass Quattro Micro triple quadrupole device (Waters, Milford, MA, USA) while mass spectrometry analysis was carried out in electrospray positive ionization mode (ESI+). The following instrumental parameters were used: source temperature and desolvation, 130 and 350 °C, respectively, the capillary voltage flow of 3.2 kV and nitrogen and desolvation gas cone of 200 and 800 L/h, respectively. The guidelines of the Commission Decision 2002/657/EC [9] and the European Commission Regulation 1881/2006/EC [10] were used for the validation and performance of the analytical method. The recovery percentages ranged between 70 and 120% in all cases. LOD and LOQ of the different compounds were as follows: LODs for AFB_1, AFB_2, FB_1 and FB_2 were 5, 2.5, 12.5, and 25 µg/kg, respectively, meanwhile LOQs for AFB_1, AFB_2, FB_1 and FB_2 were 10, 5, 25, and 50 µg/kg, respectively.

2.5 Statistical Analysis

Data obtained from both the ELISA and LC-MS/MS analysis were subjected to analysis of variance (ANOVA) and Turkey's HSD test using the SAS statistical package version 8 [11].

3. Results

3.1 Levels of Total Aflatoxins (µg/kg) and Total Fumonisins (µg/kg) in Yellow Maize at Harvest, After Drying and Storage Using ELISA Test

The detection and quantification of total aflatoxins and total fumonisins in composite samples at harvest, after drying and during storage showed that all the samples were contaminated at significantly different ($p < 0.05$) levels relative to maize maturity (Table 2).

Table 2 Total aflatoxins and total fumonisins content in composite maize samples at harvest, after drying and during storage in *Bafia* (ELISA Test).

Treatments and samples (n = 16)	Total aflatoxins (µg/kg)	Total fumonisins (µg/kg)
Harvest		
Less-mature yellow maize (80 days)	1.5 ± 0.2^{b}	10 ± 10^{b}
Semi-mature yellow maize (85 days)	1.0 ± 0.1^{a}	110 ± 20^{a}
Mature yellow maize (90 days)	1.0 ± 0.1^{a}	130 ± 10^{a}
Drying and two weeks storage		
Mature yellow maize (one week sun-drying)	1.4 ± 0.2^{a}	$5{,}170 \pm 020^{a}$
Mature yellow maize (two weeks sun-drying)	1.2 ± 0.0^{a}	70 ± 0.0^{b}
Mature yellow maize (one week barn-drying)	1.8 ± 0.1^{a}	$4{,}490 \pm 350^{a}$
Mature yellow maize (two weeks barn-drying)	1.3 ± 0.2^{a}	500 ± 70^{a}
Barn storage duration		
Mature yellow maize (one month)	1.3 ± 0.1^{a}	120 ± 30^{b}
Mature yellow maize (two months)	1.6 ± 0.1^{a}	$2{,}010 \pm 1{,}130^{a}$
Mature yellow maize (three months)	1.6 ± 0.2^{a}	$5{,}160 \pm 70^{a}$

Values in columns are means of three replicates. Numbers in a column with different superscripted letters are significantly different ($p < 0.05$) according to Turkey's HDS test. Values are mean ± SD. n = composite samples. LOD total fumonisins: < 10 µg/kg; LOD total aflatoxins: < 0.1 µg/kg; LOQ total fumonisins: 10 µg/kg; LOQ total aflatoxins: 0.1 µg/kg.

The results at harvest showed both contamination by total aflatoxins (levels below 2 µg/kg) and total fumonisins (levels below 200 µg/kg) in all samples. The levels of total aflatoxins in mature yellow maize samples after one and two weeks of sun-drying or barn-drying and storage for two weeks showed no significant difference ($p > 0.05$). However, total fumonisins contamination after two weeks of sun- or barn-drying and storage for two weeks was less than after one week. Quantitative analyses of total aflatoxins in composite samples of mature yellow maize after one, two and three months of storage in a barn showed no significant difference ($p > 0.05$). Conversely, there was a significant difference ($p < 0.05$) in the levels of total fumonisins found after the storage periods in the same samples analysed with concentration of above recommended maximum level of 4,000 µg/kg (unprocessed) in maize after three months of storage.

3.2 Reduction of Toxin Levels in Maize Products During Processing

Table 3 shows the levels of total aflatoxins and total fumonisins in yellow maize before and after processing into the different dishes and calculated percentage of toxins reduction. The initial total aflatoxin levels (1-3 µg/kg) in maize used for processing the maize products such as maize beer, maize *fufu*, maize vegetables, maize milk, roasted maize, boiled maize, maize fritters and fried maize with groundnuts were not statistically different ($p > 0.05$). On the other hand, these levels significantly differed from those (19-20 µg/kg) found in maize for processing dry or fresh maize cake with vegetables or groundnuts.

Levels of total aflatoxins and total fumonisins in the visibly mouldy and damaged grains were particularly high. Most analyzed dishes (78.6%) had total aflatoxins levels below 2 µg/kg, while 21.4% were considered high risk foods (dry or fresh flat maize cake with vegetables of 16.8 µg/kg, dry or fresh flat maize cake with groundnuts of 18.3 µg/kg and fried maize with groundnuts of 16.8 µg/kg). The results also showed that culinary techniques used for preparing different maize-based dishes could reduce levels of total aflatoxins between 8.5 and 60.7% in almost all the dishes. On the other hand, it was found that in fried maize with groundnuts, total aflatoxin levels increased by 91.9%.

The majority of maize products (77.8%) had total fumonisin levels that were less than 1000 µg/kg, meanwhile

Table 3 Total aflatoxins and total fumonisins in composite maize samples and maize products before and after processing using ELISA test.

Maize-based dishes(n= 22)	Total aflatoxins (µg/kg)			Total fumonisins (µg/kg)		
	Before processing	After processing	Reduction %	Before processing	After processing	Reduction %
Maize beer (Kwata*)	2.9 ± 1.6[a]	1.5 ± 0.2[a]	+ 47.1	5930 ± 50[b]	4610 ± 50[b]	+ 22.1
Fresh or dry flat maize cake with vegetables (kpwindim*)	20.0 ± 0.1[b]	16.8 ± 0.1[b]	+ 15.7	190 ± 40[a]	10 ± 0.0[c]	+ 94.7
Fresh or dry flat maize cake with groundnuts (Kekumba*)	20.0 ± 0.1[b]	18.3 ± 0.8[b]	+ 8.5	190 ± 40[a]	120 ± 60[a]	+ 36.8
Maize porridge (Kenouk*)	2.2 ± 0.3[a]	0.9 ± 0.1[a]	+ 60.7	5620 ± 90[b]	620 ± 340[a]	+ 89.0
Maize fufu (Couscous) (Kepen*)	2.8 ± 0.4[a]	1.2 ± 0.1[a]	+ 56.9	5990 ± 50[b]	380 ± 40[a]	+ 93.7
Maize milk (Melek me Baazi*)	2.8 ± 0.4[a]	1.1 ± 0.1[a]	+ 59.0	5990 ± 30[b]	5600 ± 80[b]	+ 6.5
Maize vegetables (Safon* or sanga*)	1.9 ± 0.1[a]	1.62 ± 0.3[a]	+ 13.4	160 ± 50[a]	110 ± 20[a]	+ 31.2
Roasted maize (Baazihangue*)	1.2 ± 0.1[a]	0.8 ± 0.1[a]	+ 38.7	< LOD	< LOD	NA
Boiled maize (Baazi*)	1.2 ± 0.0[a]	1.0 ± 0.1[a]	+ 16.9	< LOD	< LOD	NA
Maize fritters (Kpwaa*)	1.3 ± 0.2[a]	0.8 ± 0.1[a]	+ 40.7	290 ± 50[a]	240 ± 80[a]	+ 17.2
Fried maize with groundnuts (Baazihangue de keezoo*)	1.3 ± 0.2[a]	16.8 ± 1.5[a]	- 91.9	290 ± 50[a]	40 ± 10[a]	+ 86.2

*Vernacular names in *Bafia*. Values in columns are means of three replicates. Numbers in a column with different superscripted letters are significantly different ($P < 0.05$). Values are mean ± SD. n = composite samples. LOD total fumonisins: < 10 µg/kg; LOD total aflatoxins: < 0.1µg/kg; LOQ total fumonisins: 10µg/kg; LOQ total aflatoxins: 0.1µg/kg. NA = not applicable.

Table 4 LC-MS/MS data on AFB_1 and AFB_2 in critical composite maize and derivatives samples.

Composite maize samples (n = 4)	Aflatoxins (µg/kg)	
	AFB_1	AFB_2
Maize before processing		
Maize for cooking dry or fresh flat maize cake with groundnuts or vegetables	47	14
Derived products after processing		
Fried maize with groundnuts	25.61	13.60
Dry or fresh flat maize cake with groundnuts	< LOD	< LOD
Dry or fresh flat maize cake with vegetables	ND	ND

ND: Not Determined. LOD: 5 µg/kg for AFB_1 and 2.5 µg/kg for AFB_2. LOQ: 10 µg/kg for AFB_1 and 5 µg/kg for AFB_2. n = composite samples.

22.2% of maize-based products including maize milk (5600 µg/kg) and maize beer (4610 µg/kg) had levels that exceeded 4000 µg/kg. The estimated reduction of total fumonisins levels in different maize-based dishes showed values between 6.5 and 94.7%. The culinary techniques reduced the levels of total fumonisins detected in all samples up to 94.7% mainly in maize dishes that have a sieving phase and up to 60.7% (maize porridge) of the levels of total aflatoxins.

3.3 Data Validation of Aflatoxin and Fumonisin Levels Exceeding Regulated Levels in Contaminated Composite Samples by LC-MS/MS

The results show that the critical composite samples (samples with highest contamination levels of total aflatoxins via ELISA) were contaminated with a mixture of AFB_1 and AFB_2. The maize used to prepare the dry or fresh flat maize cake with groundnuts or vegetables was contaminated with AFB_1 and AFB_2 levels of 47 and 14 µg/kg respectively. Likewise, AFB_1 (25.61 µg/kg) and AFB_2 (13.60µg/kg) were also detected in fried maize with groundnuts. In dry or fresh flat maize cake with vegetables, AFB_1 and AFB_2 were at levels below the detection limit (5 µg/kg for AFB_1 and 2.5 µg/kg for AFB_2) (Table 4). The Fig. 1 showed the chromatogram pattern of aflotoxins AFB_2.

The results of FB_1 and FB_2 levels in the critical

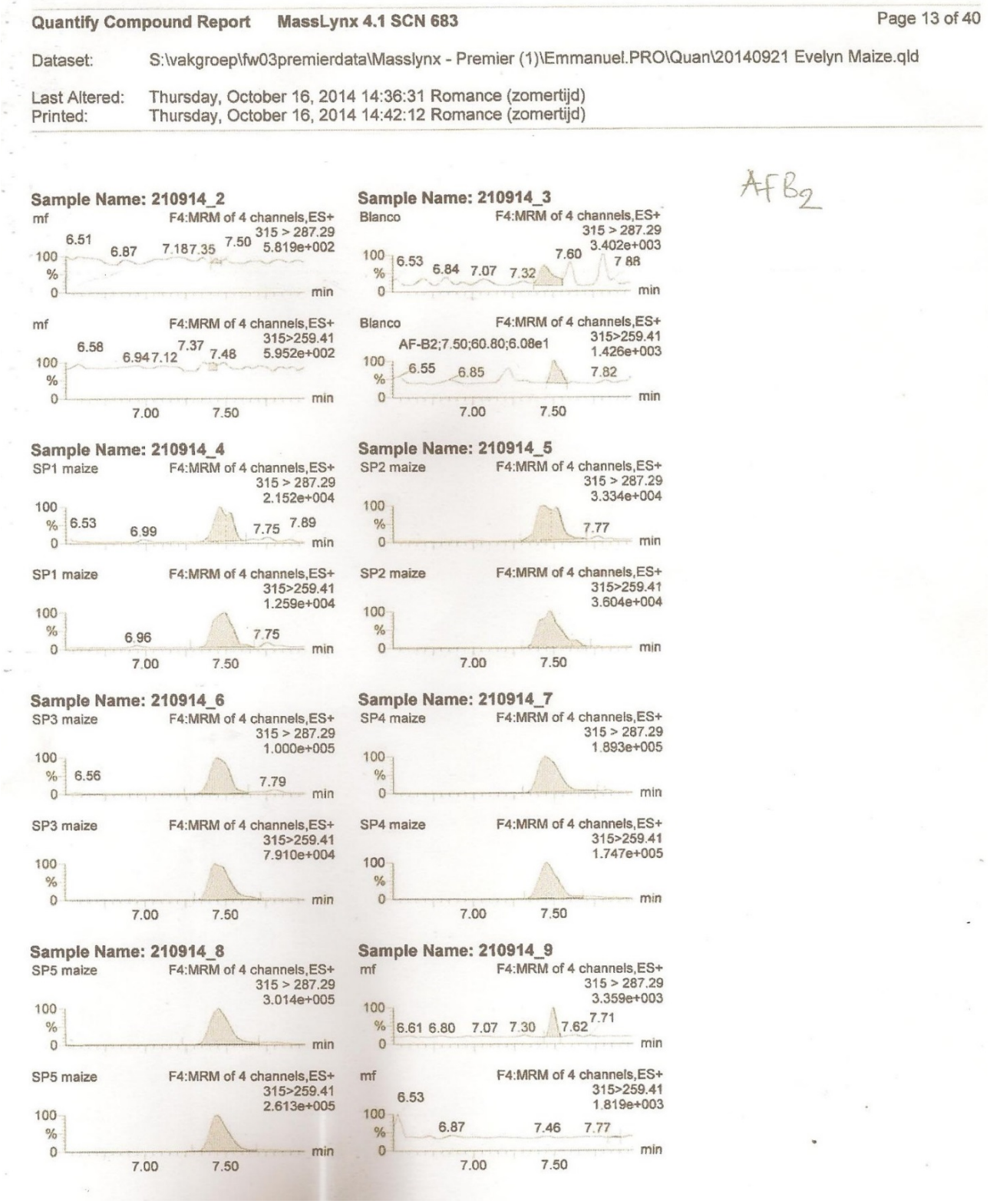

Fig. 1 Chromatogram of AFB_2 (aflatoxin B_2) by LC-MS/MS analysis.

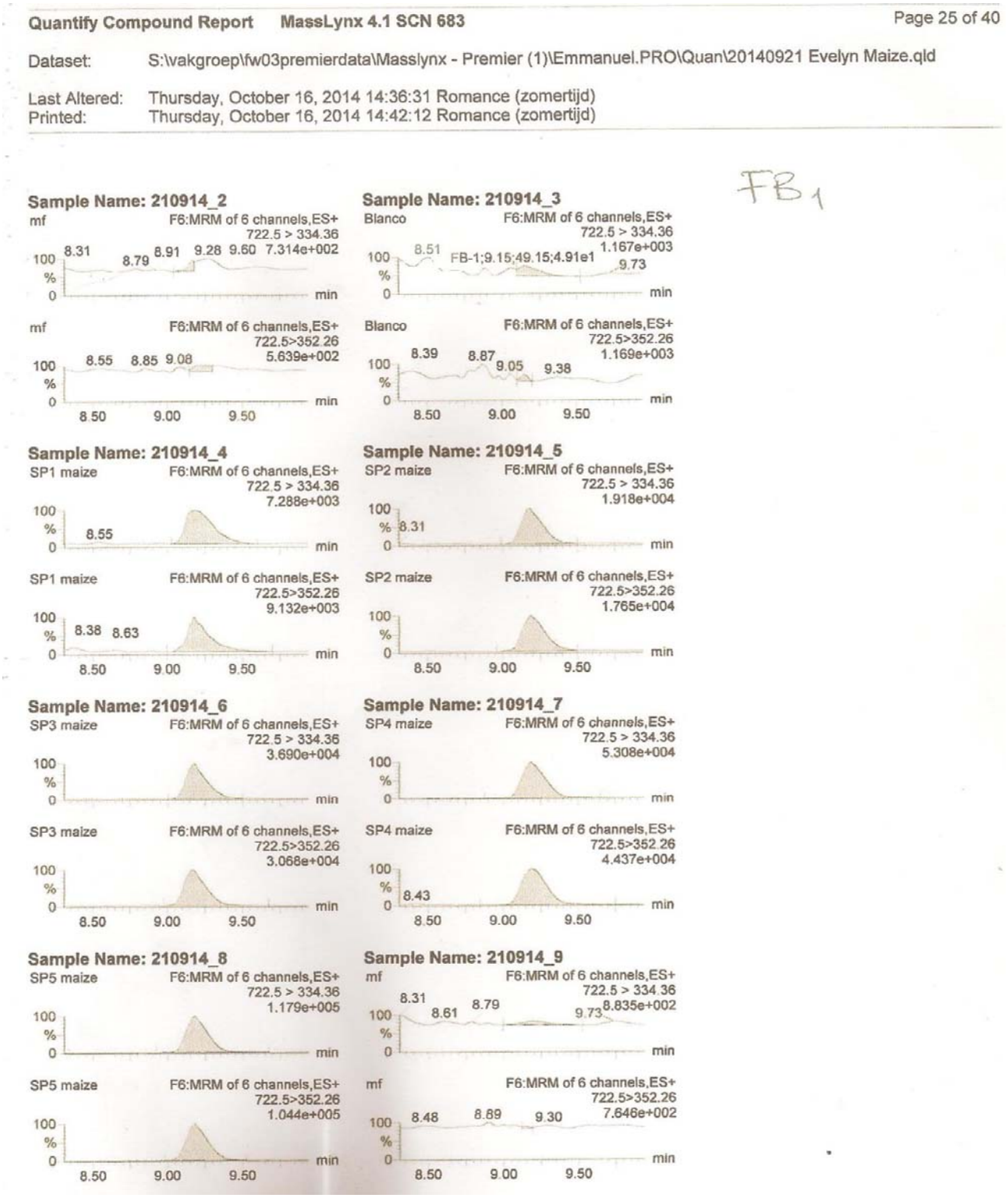

Fig. 2 Chromatogram of FB_1 (fumonisine B_1) by LC-MS/MS analysis.

Table 5 LC-MS/MS data on FB_1 and FB_2 in critical composites maize and derivatives samples.

Composite maize samples (n = 10)	Fumonisins (µg/kg)	
	FB_1	FB_2
Maize before processing		
Mature yellow maize (one week barn-drying)	2140	< LOQ
Mature yellow maize (one week sun-drying)	6990	860
Maize grains for maize porridge	230	< LOQ
Maize grains for maize milk	2140	< LOQ
Mature yellow maize (two months of storage)	2000	< LOQ
Mature yellow maize (three months of storage)	4823	448
Maize grains for maize fufu (couscous)	1960	340
Maize grains for maize beer	6990	860
Maize products after processing		
Maize milk	1490	< LOQ
Maize beer	7900	1170

LOD: 12.5 µg/kg for FB_1 and 25 µg/kg for FB_2. LOQ: 20 µg/kg for FB_1 and 50 µg/kg for FB_2. n = composite samples.

composite samples (samples with highest contamination levels of total fumonisins via ELISA) of maize and derivatives are presented in Table 5. For FB_1, the values in maize destined for processing ranged from 230 to 6990 µg/kg. In the maize milk and maize beer, FB_1 levels were 1,490 and 7,900 µg/kg respectively. In the same line, the quantitative values of FB_2 ranged between < LOQ and 860 µg/kg before processing of maize. In the products, FB_2 levels were < LOQ in maize milk and 1,170 µg/kg in maize beer (Table 5). The chromatogram pattern of fumonisins FB_1 is represented in the Fig. 2.

4. Discussion

The rate of total aflatoxins and total fumonisins differs with respect to traditional/local practice of harvest, storage, and processing. In addition, we have noticed that the environmental condition of Bafia in particular is characterized by high rainfall (4,000 mm^3/year) which is favorable for the development of fungi and secretion of aflatoxin and fumonisin. *Aspergillus* has maximum activity when moisture and temperature conditions are favorable [12]. There was no significant ($p > 0.05$) reduction in total aflatoxin levels after two weeks of sun-drying (10.3%) and barn drying (27.2%) and storage for two weeks.

The total fumonisins levels dropped by 98.7% (from 5,170 to 70 µg/kg) after two weeks of sun-drying and storage for two weeks. The highest levels (5170 µg/kg) of total fumonisins found in one week sun-dried yellow maize submitted to two weeks storage period exceeded the maximum recommended value of 4000 µg/kg in unprocessed maize and 1,000 µg/kg for direct human consumption as fixed by the European Commission amendment (1126/2007) [9]. Sun-drying for only a week particularly during rainy season is therefore a potential risk factor for maize contamination by total fumonisins.

Therefore, it is not advisable to sun-dry maize for only one week and store the grains because it will not be fully dried and so will provide for water activity suitable for fungi growth and fumonisins production. Meanwhile, after two weeks of sun-drying followed by two week storage period, the levels of total fumonisins significantly ($p < 0.05$) dropped from those found in the same samples after a week of sun-drying. As such, it is a good practice to sun-dry maize for a minimum of two weeks prior to storage to improve its safety levels from a fumonisin perspective. Likewise, these observations were the same for barn-drying as for sun-drying after one and two weeks.

Total aflatoxins contamination seems stable during storage meanwhile total fumonisins increase with

period of storage. These findings were similar to those of [3] who reported that the longer the storage time, the higher the risk of contamination by fumonisins. *Fusarium spp*. has been found as the causal agent of fumonisins contamination on maize from the forest and the western highlands of Cameroon. While poor storage methods contribute to the accumulation of mycotoxins, the presence of fumonisins may not be visible as maize may have high levels of total fumonisins yet shows a normal appearance [13]. These results are consistent with those reported by many other authors on mycotoxin contamination in maize and maize-derived products in Missisipi [1], Benin [14], Nigeria [15], and Tanzania [16].

Analysis of the results for validation showed that levels of AFB_1 are higher in all the positive composite samples compared to AFB_2. This is in agreement with other studies reported in the literature on AFB_1 contamination of foods. This also portrays AFB_1 as a prevalent toxin in foods intended for human and animal consumption [17]. The results also showed that FB_1, which is the most predominant fumonisins analogue was more abundant in maize before and after processing when compared to FB_2. Accordingly, it also reported that FB_1 continues to be more prevalent and re-occurring in food as compared to other fumonisins analogues [18]. Especially in sub-Saharan Africa (SSA), where conditions for mycotoxin proliferation are available, FB_1 continues to be a commonly encountered mycotoxin in maize and maize products [16].

The previous works in this study have revealed for the first time in Cameroon that processing maize into derivatives can reduce levels of both total aflatoxins and total fumonisins by at least 60% in the majority of products. But in fried maize with groundnuts, total aflatoxin levels increased by 91.9%. This was strange, but may be explained in that it is probable that the groundnuts used in the preparation of this dish were contaminated with total aflatoxins and thus the main source of increased total aflatoxins in the final product [7].

Future studies plan to verify this aspect by analysing other ingredients to be use in the preparation of the dish. Although no study has reported an increase in mycotoxin levels after frying in the literature, *masa* fried at 140-170 ^{o}C gave no reduction in fumonisins [19]. Bullerman and Bianchini [20] also suggested that dry heat appears to be more destructive to mycotoxins than moist heat, which was used in this study. Indeed, in this study, the total aflatoxins contamination before processing was significantly ($p < 0.05$) higher compared to their contents in different dishes except the fried maize with groundnuts after cooking. This difference could be partly due to the cooking time and supplemented material used which vary according to different dishes as well as other food habits and sanitary adopted during food preparation. In addition, the presence of proteins, the pH and the treatment by removing broken particles, can significantly reduce the concentration of total aflatoxins in finished products [21]. The highest amount of total fumonisins and total aflatoxins is in the seed coat which is removed prior to or during sieving or aspiration thus, reduces the degree of contamination at least seven folds [22]. Meanwhile, in dishes with more maize and groundnuts, the levels may sometime increase as poorly preserved groundnuts are a favorable habitat for the development of *Aspergillus* and the secretion of aflatoxins, which are thermostable during traditional cooking temperatures in Bafia [7]. Some operations linked to the preparation of maize dishes were found to have insignificant reduction potential against the studied mycotoxins. These traditional food processing techniques may therefore not potentially be useful in detoxifying mycotoxin contaminated foods.

5. Conclusions

This investigation on mycotoxin levels in maize and maize-based foods in Bafia (Centre Cameroon) as influenced by harvest, storage habits and processing

techniques clearly demonstrated that total aflatoxins and total fumonisins presented in some of the composite samples exceed levels that established by regulatory agencies bodies. Post-harvest practices and food processing habits could largely increase the levels of these toxins in the final product, while certain culinary practices could also substantially reduce the levels. This pilot study is useful for regulatory action, control and monitoring total aflatoxin and total fumonisin levels in foods in Cameroon

Conflict of Interest

None

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