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Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin

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Abstract

The fate of aflatoxins and fumonisins, two mycotoxins that cooccur in maize, was studied through the traditional processing of naturally contaminated maize in *mawe*, *makume*, *ogi*, *akassa*, and *owo*, maize-based foods common in Benin, West Africa. Levels of total aflatoxin and fumonisin were measured at the main unit operations of processing, and the unit operations that induce significant reduction of mycotoxin level were identified. Overall reduction of mycotoxin level was more significant during the preparation of makume (93% reduction of aflatoxins, 87% reduction of fumonisins) and akassa (92% reduction of aflatoxins, 50% reduction of fumonisins) than that of owo (40% reduction of aflatoxins, 48% reduction of fumonisins). Sorting, winnowing, washing, crushing combined with dehulling of maize grains were the unit operations that appeared very effective in achieving significant mycotoxin removal. Aflatoxins and fumonisins were significantly recovered in discarded mouldy and damaged grains and in washing water. Fermentation and cooking showed little effect. During the preparation of ogi and akassa, reduction of fumonisin levels measured in food matrix was lower (50%) compared to mawe and makume, probably due to significant fumonisin release in ogi supernatant. Consequently, the use of ogi supernatant for preparing beverages or traditional herbal medicines could be harmful as it is likely to be contaminated with mycotoxin from the raw maize.

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Keywords: Maize; Maize-based products; Processing; Aflatoxins; Fumonisins; Benin

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1. Introduction

Aflatoxins and fumonisins are metabolites respectively produced by toxigenic species of *Aspergillus* and *Fusarium*, of which *Aspergillus flavus* and *Aspergillus parasiticus* (Pittet, 1998) and *Fusarium verticillioides* and *Fusarium proliferatum* (Keller et al., 1997) are by far the most important. Attention is increasingly given to these mycotoxins for several reasons. They have been shown to be directly responsible for several animal diseases. Aflatoxins are found to be hepatotoxic and potent hepatocarcinogens in animals (Wogan, 1992). Fumonisins have been shown to be the causative agents of leukoencephalomalacia in horses (Kellerman et al., 1990) and pulmonary edema syndrome in pigs (Harrison et al., 1990).

Aflatoxins and fumonisins are known to be hazardous to the health of humans, in some cases directly causing illness and even death. Aflatoxins are implicated in liver cancer (JECFA, 1998; Wild and Hall, 2000). Chao et al. (1991) reported an incident when aflatoxins present in a foodstuff consumed by people in Malaysia in 1988 were strongly implicated as the cause of death of 13 children. Aflatoxins have been reported to impair childhood growth in children from Benin and Togo (Gong et al., 2002). Fumonisins were reported to be associated with oesophageal cancer in rural areas in South Africa (Rheeder et al., 1992) and China (Chu and Li, 1994) and liver cancer in China (Ueno et al., 1997). Consumption of mouldy sorghum and maize containing fumonisin B₁ has been associated with an outbreak of abdominal pain and diarrhoea in India (Bhat et al., 1997).

Aflatoxins and fumonisins occur worldwide in maize, either alone or together (Kpodo et al., 2000), maize being a dietary staple food in many countries in the world (Thiel et al., 1996). Per capita daily consumption of maize averages more than 246 g in Benin (Hounhouigan et al., 1999). Maize is consumed in different forms in the world. Nago et al. (1997) reviewed traditional African maize products and found various forms including porridges, pastes, dumplings, cakes, fritters, and beverages.

Effects of processing on mycotoxin contamination in food products are increasingly being investigated throughout the world, and this strategy is showing great promise for mycotoxin reduction. The use of physical methods, including cleaning, separation of screenings, washing, aqueous extraction, dehulling and milling, has been shown to be effective, to a certain extent, in reducing mycotoxins in cereals (Charmley and Prelusky, 1995; Voss et al., 1996; Shetty and Bhat, 1999). Aflatoxin and fumonisin levels in tortillas were found to be significantly reduced due to alkaline cooking (Dombrink-Kurtzman et al., 2000; Voss et al., 2001).

There is very little information concerning attempts to reduce mycotoxin contamination in maize using traditional processing methods in Africa, although many maize-based food products exist. In Benin, about 40 different maize processing methods have been recorded (Nago, 1997). This research was carried out to determine the fate of both aflatoxins and fumonisins in naturally contaminated maize products and to identify operations that give a significant reduction in mycotoxin levels during processing.

Three traditional maize-based products commonly consumed in West Africa were used in this study. These were *mawe*, *ogi* and *owo*. Mawe is a solid-state fermented dough used in Benin, Togo, and Nigeria for cooking several dishes (Hounhouigan et al., 1993). One popular dish from mawe is *makume*, a thick paste consumed with stew. Ogi is a gruel obtained by fermentation of a suspension of wet-milled maize in water, processed into *akassa*, another thick paste and also consumed with stew (Hounhouigan, 1994). It is also popular in West African countries. Owo is a nonfermented thick paste obtained from whole maize meal named *lifin*. It is a common and popular food in many African countries (Nago et al., 1997).

2. Material and methods

2.1. Origin of maize samples

Maize, in three separate samples collected from the same store, was obtained from the research station of Ina, situated in the North of Benin. This was of the 90-day cultivar DMR-ESR-W, an improved IITA white variety. DMR-ESR-W is resistant to downy mildew (*Peronosclerospora sorghi*) and to maize streak virus (Schulthess et al., 2002).

2.2. Preparation of maize products

Preparation of mawe, ogi, and lifin (whole maize meal) into derived products, namely, makume, akassa, and owo, respectively, was executed using the expertise of four women, experienced in the production of these products.

Mawe was prepared following the traditional procedure indicated in Fig. 1 as described by Hounhouigan et al. (1993). Three replicates of 5 kg of maize (total fumonisin level= $1.99\pm0.06 \mu g/g$, and total aflatoxin level=15.28±0.32 ng/g) were sorted, winnowed, and washed. Sorting consisted in removing visibly mouldy, insect-damaged and broken grains by hand. Winnowing, a complementary process to sorting, consisted in discarding the rest of impurities present in the sorted maize. Thus, a certain quantity of sorted maize was collected in a circular metallic tray and thrown into the air by the operator allowing impurities and small broken grains to be blown away. Maize washing came after sorting and winnowing and included thoroughly rubbing the grains in water for 15 min and removing all upper floating grains and impurities. Grains were washed in water in the ratio 1:2 (w/v).

The clean grains were crushed with a plate disc mill (AMUDA, India), passed through a 2×2 mm

plastic sieve, and sieved with a 0.7×0.7 mm metallic sieve to obtain separately grits, hulls, and a fine fraction (fines). The grits were washed by rubbing them by hand for a few minutes (10-15 min) in water, and then a further soaking in the water for about 2 h in the ratio 1:3 (w/v). Meanwhile, the embryo and remaining hulls were discarded and added to the hulls previously collected as waste during screening. The washed grits and fines were mixed and the mixture finely ground with the plate disc mill. The resulting meal was kneaded while adding water to obtain a dough (mawe). The mawe, placed in a container covered with a polyethylene sheet, was allowed to ferment at room temperature (28–30 °C), one subsample for 24 h and the second one for 72 h. Fermented mawe was then cooked into makume. Cooking consisted in making initially a suspension of mawe (500 g) in water (250 ml). This suspension was then intermittently added, in small quantities (approximately 200 g), to boiling water (1250 ml) and stirred until a desired consistency was achieved. Cooking process lasted 25-30 min.

Ogi was prepared following the traditional processing method (Fig. 2) described by Nago (1997). Three replicates of 5 kg of maize (total fumonisin level= 3.35 ± 0.05 µg/g, and total aflatoxin level

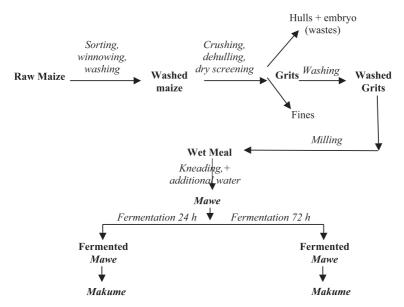


Fig. 1. Flow diagram outlining the preparation of mawe and makume, two maize-based foods of Benin.

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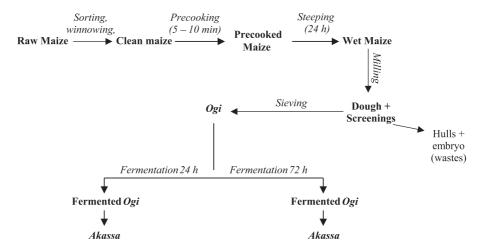


Fig. 2. Flow diagram outlining the preparation of ogi and akassa in Benin.

≥22.00±0.26 ng/g) were sorted, winnowed, not washed, and precooked for 10 min at 90–100 °C. The precooked maize was steeped in water in the ratio 1:3 (w/v) for 24 h, milled in a plate disc mill, and sieved in water in the ratio 1:5 (w/v) with a muslin cloth to discard hulls and embryo. The resulting dough (ogi) underwent a fermentation process, one subsample for 24 h and the second one for 72 h. The fermented ogi was then cooked into akassa, a fermented paste like makume obtained from mawe. The cooking of ogi into akassa followed the same procedure as described above.

Owo was prepared following the traditional processing method (Fig. 3). Three replicates of maize (5 kg; total fumonisin level= 2.89 ± 0.08 µg/g, and total aflatoxin level $\geq 22.00\pm0.26$ ng/g) were sorted, winnowed, not washed, and milled in a plate disc mill to obtain lifin (maize meal). A suspension of lifin (250 g of lifin added to 1000 ml of boiling water) was cooked with stirring to obtain owo. Cooking process lasted 20 min.

2.3. Laboratory analyses

Moisture content of maize samples was determined at each step in the processing of each product. Three replicate samples of 5 g of each were dried to a constant weight in a forced-air oven (Memmert, Germany) at 105 °C for 24 h (AACC, 1986). Fermented mawe and ogi were sampled after 0, 24, and 72 h of fermentation, and pH was measured.

Total fumonisin content was determined in samples collected at different steps of processing as indicated in the flow diagram for each product (Figs. 1, 2 and 3) using the VICAM method (VICAM Science Technology, 1998). Ground maize (50 g) was mixed with 5 g of sodium chloride and 100 ml of methanol/water (80:20). The mixture was blended at high speed for 1 min using a blender (Waring Commercial, Torringston, USA) and then filtered through a fluted filter paper. The extract (10 ml) was diluted with 40 ml of phosphate-buffered saline (PBS)/0.1% Tween-20 wash buffer and filtered through a 1.0 μm microfibre filter. The diluted extract was then passed through the immunoaffinity column (FumoniTest[™] column, VICAM, Watertown, USA), which was washed with 10 ml of PBS/0.1% Tween-20 wash buffer followed



Fig. 3. Flow diagram outlining the preparation of owo in Benin.

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by 10 ml of PBS. Fumonisins were eluted from the column with 1 ml HPLC grade methanol. A mixture of Developer A and Developer B (1 ml) was added to the elute and collected in a cuvette. Fumonisin levels were determined with a fluorometer (VICAM Fluorometer Series 4).

Total aflatoxin content in maize was measured using the AflaTest immunoaffinity column (AflaTest™ column, VICAM). Sample preparation was performed using the Fumonitest protocol. Before aflatoxin measurement, a mixture of 1 ml of Aflatest Developer A and 9 ml of sterilised distilled water was added to the column elute. Levels of both aflatoxin and fumonisin in each sample, initially measured on wet basis, were calculated on dry basis.

Total aflatoxin and fumonisin measurement was performed during processing on the following intermediate products:

- Mawe production: raw maize, washed maize, mouldy and damaged grains, washed grits, hulls, fines, mawe, fermented mawe, and makume.
- Ogi production: raw maize, clean maize, mouldy and damaged grains, dough+screenings, hulls, ogi, fermented ogi, and akassa.
- Owo production: raw maize, clean maize, mouldy and damaged grains, lifin (maize meal) and owo.

Because maize cleaning was the stage that gave more reduction in mycotoxin level during the three processings, mycotoxin content was analysed at each step of this operation in order to get more precision and to determine whether aflatoxin and fumonisin passed in the disposable fractions. Three replicates of 2 kg of maize (total fumonisin level= $4.80\pm0.32 \mu g$ / g, and total aflatoxin level=6.57±0.26 ng/g) were then sorted, winnowed, and washed following the method described with the preparation of mawe. Aflatoxin and fumonisin levels were measured in maize after sorting and winnowing, washed maize, discarded fractions, and washing water. The discarded fractions consisted of visibly mouldy and damaged grains, and pericarps obtained after sorting and winnowing, and upper floating grains collected during washing. Mycotoxin levels in each intermediate maize product, initially calculated on dry basis, were related to that in the washing water using the same mass.

2.4. Statistical analyses

Statistical analyses were performed using SPSS for Window version 10.0 (SPSS, Chicago, Illinois). Analysis of variance (ANOVA) and Tukey's HSD test were used to compare the means of the total aflatoxin and fumonisin levels measured in samples collected at different processing steps. Mean total aflatoxin and fumonisin were transformed to $\log(x+1)$ before analyses, but the data are presented untransformed.

3. Results

3.1. Fate of aflatoxin and fumonisin during preparation of mawe and makume

Total aflatoxin and fumonisin levels in maize products during the preparation of mawe and makume are shown in Table 1. Both aflatoxin and fumonisin significantly decreased during the processing (p<0.01). Total aflatoxin level decreased from 15.28 ng/g in the raw maize to a nondetectable level in mawe. Aflatoxin was not detected in makume. A 91% reduction was already observed after sorting, winnowing, and washing of the raw maize. No aflatoxin was detected in discarded hulls, embryo and in fines (screenings).

Total fumonisin in maize followed the same trend as that for aflatoxin (Table 1). Levels decreased from 1.99 $\mu g/g$ in the raw maize to not being detected in mawe. Fumonisin was not detected in makume (Table 1). Initial maize cleaning induced a significant reduction of 74% of fumonisin in maize. In contrast to aflatoxin, 0.41 and 0.38 $\mu g/g$ of fumonisin were detected in the discarded hulls, embryo and in the fines, respectively.

Neither aflatoxin nor fumonisin was detected in the nonfermented mawe, and consequently assessment of fermentation effect was not possible (Table 1). The assessment of cooking effect during the preparation of makume was not possible as well, as the toxins were at undetectable levels.

Analysis of mycotoxin content at each step of the initial process of maize cleaning showed meaningful levels of aflatoxin and fumonisin in the disposable fractions consisting of visibly mouldy and damaged

Table 1
Mean total aflatoxin and fumonisin levels in maize products at each processing stage during the preparation of mawe (dry basis)

Maize products	Dry matter content (%)	рН	Mean total aflatoxin (ng/g)		Mean total fumonisin (μg/g)	
Raw maize	84.51 ± 0.06		15.28 ± 0.32	a	1.99 ± 0.06	a
Washed maize	84.35 ± 0.04		1.42 ± 0.06	b	0.54 ± 0.03	b
Washed grit	55.90 ± 0.32		nd	c	nd	e
Mawe	54.58 ± 0.27	5.90 ± 0.14	nd	c	nd	e
Fermented mawe 24 h	53.02 ± 0.28	4.14 ± 0.08	nd	c	nd	e
Fermented mawe 72 h	51.01 ± 0.32	3.70 ± 0.10	nd	c	nd	e
Makume 24 h	39.01 ± 0.24		nd	c	nd	e
Makume 72 h	40.17 ± 0.34		nd	c	nd	e
Hulls+embryo	82.99 ± 0.17		nd	c	0.41 ± 0.15	c
Fines (screenings)	83.29 ± 0.21		nd	c	0.37 ± 0.06	d

Values in columns are means of three replicates.

Means in the same column followed by the same letter are not significantly different (p>0.05) according to Tukey's HSD test. nd: Not detected, level <1 ng/g for aflatoxins; level <0.25 μ g/g for fumonisins.

grains, upper floating grains collected during washing and washing water (Table 2). Aflatoxin and fumonisin levels were particularly high in the mouldy and damaged grains (11.73 ng/g and 8.83 μ g/g, respectively).

3.2. Fate of aflatoxin and fumonisin during the preparation of ogi and akassa

Both aflatoxin and fumonisin levels significantly decreased (p<0.01) during the production of ogi and akassa (Table 3). An 80% reduction (Table 4) of the total aflatoxin level was observed from the raw maize to ogi (from \geq 22.00 to 4.50 ng/g). Aflatoxin was detected in akassa, but the level was quite low (1.83 ng/g), corresponding to a significant reduction of about 92% (Table 4). Initial maize cleaning in this case resulted in an aflatoxin reduction of 61%. A significant level of aflatoxin (7.55 ng/g) was detected

in the discarded hulls and embryo. This was about 34% of the level found in the raw maize.

Total fumonisin level also decreased during processing, but less than in the case of aflatoxin (Table 3). A 29% decrease (Table 4) was observed from the raw maize to ogi (from 3.35 to 2.37 μ g/g). Fumonisin level in akassa was 1.74 μ g/g (Table 3), corresponding to about 48% of the level in the raw maize (Table 4). A fumonisin level of 2.35 μ g/g was detected in the discarded hulls and embryo.

Fermentation gave rise to significant differences in aflatoxin levels in fermented and nonfermented ogi (p<0.05), with 18% reduction between the latter and the former product. This difference however was not significant in the case of fumonisin, although there was a reduction of 13% (p>0.05). No significant differences were found between the aflatoxin and fumonisin levels in ogi whether fermentation of this product lasted 24 or 72 h (p>0.05).

Table 2
Mean total aflatoxin and fumonisin levels in maize products and disposable fractions during maize cleaning process

Products	Dry matter content (%)			Mean total fumonisin (μg/g)		
Raw maize	83.80 ± 0.38	6.57 ± 0.26	b	4.80 ± 0.32	b	
Maize after sorting and winnowing	84.01 ± 0.43	2.67 ± 0.20	c	1.50 ± 0.10	c	
Washed maize	80.80 ± 0.77	1.87 ± 0.09	cd	1.27 ± 0.07	c	
Discarded mouldy and damaged grains	82.70 ± 0.35	11.73 ± 0.83	a	8.83 ± 1.60	a	
Washing water	_	1.00 ± 0.05	d	0.76 ± 0.83	c	

Values in columns are means of three replicates.

Means in the same column followed by the same letter are not significantly different (p>0.05) according to Tukey's HSD test. Mycotoxin levels are calculated using the same mass of intermediate maize products and washing water.

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Table 3
Mean total aflatoxin and fumonisin levels in maize products at each processing stage during the preparation of ogi (dry basis)

Maize products	Dry matter content (%)	pH	Mean total aflatoxin (ng/g)		Mean total fumonisin (μg/g)	
Raw maize	84.19±0.07		22.00±0.26	a	3.35±0.05	a
Steeped maize	84.21 ± 0.06		8.62 ± 0.09	b	2.76 ± 0.11	b
Dough+screenings	43.65 ± 0.57		7.79 ± 0.12	c	2.75 ± 0.09	bc
Ogi	42.10 ± 0.47	6.20 ± 0.10	4.50 ± 0.20	d	2.37 ± 0.22	cd
Fermented ogi 24 h	40.40 ± 0.61	4.00 ± 0.10	3.69 ± 0.03	e	2.07 ± 0.12	d
Fermented ogi 72 h	39.97 ± 0.48	3.05 ± 0.15	3.65 ± 0.05	e	2.28 ± 0.14	d
Akassa 24 h	43.79 ± 0.32		3.72 ± 0.02	e	2.18 ± 017	d
Akassa 72 h	36.56 ± 0.29		1.83 ± 0.02	f	1.74 ± 0.07	e
Hulls+embryo	42.19 ± 0.19		7.55 ± 0.43	c	2.35 ± 0.03	d

Values in columns are means of three replicates.

Means in the same column followed by the same letter are not significantly different (p>0.05) according to Tukey's HSD test.

Cooking 24-h-fermented ogi to akassa did not significantly affect the aflatoxin or fumonisin content. In contrast, mycotoxin levels were significantly lower (1.83 ng/g for aflatoxins, 1.74 μ g/g for fumonisins) in the akassa from 72-h-fermented ogi than in the akassa from 24-h-fermented ogi (3.72 ng/g for aflatoxins, 2.18 μ g/g for fumonisins; p<0.05).

3.3. Fate of aflatoxin and fumonisin during the preparation of owo

The preparation of owo had a significant effect on mycotoxin levels (Table 5). A significant decrease in aflatoxin levels was observed from the raw maize (\geq 22 ng/g) to lifin (maize meal; 12.62 ng/g; p<0.01), with a meaningful reduction of 37% after initial maize cleaning prior to milling. No further significant reduction occurred during cooking of maize meal to owo.

Table 4
Mean percentage of reduction in total aflatoxin and fumonisin levels during maize processing

Maize processing	Mean percentage of mycotoxin reduction (%)			
	Total aflatoxin	Total fumonisin		
Raw maize → mawe	≥91	≥87		
Raw maize → mawe	≥93	≥87		
→ makume				
Raw maize → ogi	80	29		
Raw maize → ogi	92	48		
→ akassa				
Raw maize → owo	40	48		

Fumonisin decreased from 2.89 μ g/g in raw maize to 1.45 μ g/g in maize meal, with a 45% reduction after initial maize cleaning. Also in this case, cooking maize meal to owo did not significantly reduce the fumonisin level (p>0.05).

4. Discussion

Results of this study have shown that processing maize into traditional products can significantly reduce levels of both aflatoxin and fumonisin up to 93%. This indicates that elimination of mycotoxins in naturally contaminated maize is, to a certain extent, possible using such food processing techniques. Reduction of mycotoxins was more substantial during the production of makume (mawe) and akassa (ogi) than in the preparation of owo. This might be due to the fact that techniques for making makume and

Table 5
Mean total aflatoxin and fumonisin levels in maize products at each processing stage during the preparation of owo (dry basis)

Maize products	Dry matter content (%)	Mean total aflatoxin (ng/g)		Mean total fumonisin (μg/g)	
Raw maize	84.67±0.02	22.00 ± 0.26	a	2.89 ± 0.08	a
Clean maize	84.62 ± 0.04	13.79 ± 0.19	b	1.61 ± 0.09	b
Maize meal	86.15 ± 0.10	12.62 ± 0.43	c	1.38 ± 0.02	c
Owo	70.82 ± 0.18	13.13 ± 0.25	bc	1.45 ± 0.05	c

Values in columns are means of three replicates.

Means in the same column followed by the same letter are not significantly different (p>0.05) according to Tukey's HSD test.

akassa involve critical steps for mycotoxin reduction. Shephard et al. (2002) also found a low reduction of fumonisin level (23%) in South African stiff porridge, which has a method of processing similar to that of owo.

Some operations linked to the preparation of these maize-based foods were found to have been very effective in significantly reducing mycotoxins. The most important has been the simple cleaning, which consisted in sorting, winnowing and/or washing the contaminated maize grains, as reduction of mycotoxins (up to 91%) mostly occurred during this stage. A high amount of aflatoxin and fumonisin was found in the discarded mouldy and damaged grains during sorting, as well as in the upper floating grains collected during washing. This confirms findings of previous studies (Rheeder et al., 1992; Desjardins et al., 1998), and suggests that systematic disposal of all visibly mouldy and damaged grains and impurities is a very useful detoxification operation. Sydenham et al. (1994) previously found that removal of screenings from maize bulk significantly reduced fumonisin levels. However, hand-sorting visibly mouldy grains with the aim of substantially reducing mycotoxin levels is likely to depend on the ability of the people responsible for this activity. People trained to easily recognise diseased grains are apparently more efficient at achieving this goal (Desjardins et al., 2000).

The role of maize washing during the cleaning process was not neglectible as a significant quantity of aflatoxin and fumonisin was detected in the washing water. Shetty and Bhat (1999) found substantial amounts of fumonisin (up to 74%) to be removed by simply washing maize grains, immersing them in water, and by removing the upper floating fraction. While sensibilising populations on decontamination of food products during processing, it is very important to stress on operations such as sorting and washing.

Crushing and dehulling maize during mawe production has been also identified as another critical operation for mycotoxin reduction by removing the grain pericarp and embryo. In this study, recovery of fumonisin for instance in maize after crushing and dehulling was almost negligible. This finding provides additional evidence that removal of pericarps and embryo of maize grains, mechanically or chemi-

cally, can also play an important role in the reduction of fumonisins in naturally contaminated maize (Sydenham et al., 1995; Canela et al., 1996; Katta et al., 1997; Voss et al., 2001).

The crushing and dehulling of maize did not influence aflatoxin contamination during the preparation of mawe, because no toxin was detected in the discarded hulls and embryo. Aflatoxin levels were already so low in the cleaned grains that levels in the hulls and embryo were below detection. In the case of ogi, a significant level of aflatoxin was, however, recovered in the discarded hulls and embryo after sieving. The aflatoxin levels were still so high in the dough obtained after milling, prior to sieving, that meaningful quantities are likely to be detected in the hulls and embryo. This suggests that aflatoxin distribution in maize fractions during processing may be influenced by contamination levels (Lopez-Garcia and Park, 1998).

Fermentation did not appear to have a significant impact on the levels of mycotoxins in products considered in this study. Only 18% and 13% reduction of aflatoxin and fumonisin levels, respectively, were observed during fermentation of ogi. Even prolonging the fermentation time from 24 to 72 h did not significantly affect mycotoxin levels in maize products. Previous studies have presented similar results (Kpodo et al., 1996; Desjardins et al., 2000; Kpodo et al., 2000). Kpodo et al. (1996) explained persistence of aflatoxin during the fermentation process by the presence of aflatoxin precursors in maize grains. These researchers also suggested that in acid condition, there is no reduction of aflatoxin but rather a reformation of the toxin. Reduction of pH during fermentation together with the presence of organic acids produced by the organisms present in the fermented product are likely to create such condition. In this study, microflora of fermentation has not been analysed, but previous works in Benin found lactic acid bacteria (mainly Lactobacillus spp.) and yeasts (mainly Candida spp.) to be the dominating microorganisms isolated in both mawe (Hounhouigan, 1994) and ogi (Nago et al., 1998). All these organisms grow together in the fermented product contributing to production of organic acids. The pH reduced during fermentation of ogi in this work, from 6.20 in nonfermented ogi to 4.00 in ogi 24 h fermentation and to 3.05 in ogi 72 h fermentation.

There was no evidence that cooking has a significant effect on the reduction of mycotoxins during the production of makume, akassa, or owo. In all cases, cooking did not last more than 30 min and is therefore unlikely to significantly reduce mycotoxin levels. Kpodo et al. (1996) found that ordinary cooking of fermented maize dough for a 3-h period resulted in up to 80% reduction of aflatoxin level. These authors suggested that degradation of aflatoxin during the cooking process might be favoured by moist conditions. Further work needs to be conducted in order to provide more information on interactions between fermentation, pH, and cooking temperature. More recently, Shephard et al. (2002) using commercial maize meal, found a reduction of fumonisin levels of 23%, after only 20 min of cooking. Aflatoxin and fumonisin are usually reported to be heat-stable and are not easily destroyed by ordinary cooking (Alberts et al., 1990; Sinha, 1998), except at high temperatures (more than 150°C for fumonisin; Bolger et al., 2001). However, aflatoxin can be partially removed by cooking, especially when this occurs under pressure in moist conditions (Sinha, 1998).

Another processing step probably responsible for significant mycotoxin reduction was the way the ogi supernatant was used during the cooking of akassa. The supernatant from the 72-h-fermented ogi was replaced with simple water and discarded or used to produce beverages when the acidity levels were inordinately high to give an acceptable akassa. This may explain why both aflatoxin and fumonisin contents were reduced in akassa prepared from 72-hfermented ogi. Here, mycotoxins probably diffused in the ogi supernatant, which was discarded. This observation is in agreement with the findings of Canela et al. (1996), who found fumonisin B₁ to migrate from contaminated maize grains to the steeping water after 48 h. While discarding ogi supernatant constitutes a significant decontamination process, its use to prepare beverages, which is a common practice in West Africa, may be harmful to consumers and should be discouraged.

5. Conclusion

Some traditional food processing techniques in Africa may potentially be useful for detoxifying foods

from mycotoxin contamination. Operations such as systematic discarding mouldy and damaged foods and washing before processing are more likely to reduce considerable levels of mycotoxins. There is however increasing evidence that mycotoxin molecules, specifically fumonisin, bind with starch to form a complex that cannot be detected (Bullerman and Tsai, 1994; Kim et al., 2002). Alternatively, they react with reducing sugars, such as D-glucose to give sugar adducts (Howard et al., 1998; Seefelder et al., 2001; Voss et al., 2001) or are hydrolysed to the aminopolyols AP₁ and AP₂ (Dombrink-Kurtzman et al., 2000; Voss et al., 2001). Further research is therefore needed to clarify whether the mycotoxins apparently lost during the preparation of foods are really destroyed, hydrolysed, or bound to the food matrix to become nonrecoverable.

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References

AACC, 1986. Approved methods of the American Association of Cereal Chemists. St. Paul, MN.

Alberts, J.F., Gelderblom, W.C.A., Thiel, P.G., Marasas, W.F.O., Van Schalkwyk, D.J., Behrend, Y., 1990. Effects of temperature and incubation period on production of fumonisin B₁ by Fusarium moniliforme. Applied and Environmental Microbiology 56, 1729–1733.

Bhat, R.V., Shetty, P.H., Amruth, R.P., Sudershan, R.V., 1997. A foodborne disease outbreak due to the consumption of moldy sorghum and maize containing fumonisin mycotoxins. Clinical Toxicology 35, 249–255.

Bolger, M., Coker, R.D., DiNovi, M., Gaylor, D., Gelderblom, W., Olsen, M., Paster, N., Riley, R.T., Shephard, G., Speijers, G.J.A., 2001. Fumonisins. In: Safety Evaluation of Certain Mycotoxins in Food, WHO Food Additives Series 47, FAO Food and Nutrition Paper 74, Prepared by the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO, Geneva, pp. 103–279.

Bullerman, L.B., Tsai, W.Y.J., 1994. Incidence and levels of Fusarium moniliforme, Fusarium proliferatum and fumonisins

- in corn and corn-based foods and feeds. Journal of Food Protection 57, 541-546.
- Canela, R., Pujol, R., Sala, N., Sanchis, V., 1996. Fate of fumonisin B₁ and B₂ in steeped corn kernels. Food Additives and Contaminants 13, 511-517.
- Chao, T.C., Maxwell, S.M., Wong, S.Y., 1991. An outbreak of aflatoxicosis and boric acid poisoning in Malaysia: a clinicopathological study. Journal of Pathology 164, 225–233.
- Charmley, L.L., Prelusky, D.B., 1995. Decontamination of Fusarium mycotoxins. Applied and Environmental Microbiology 6, 421–435.
- Chu, F.S., Li, G.Y., 1994. Simultaneous occurrence of fumonisin B₁ and other mycotoxins in moldy corn collected from the People's Republic of China in region with high incidence of oesophageal cancer. Applied and Environmental Microbiology 60, 847–852.
- Desjardins, A.E., Plattner, R.D., Lu, M., Claflin, L.E., 1998. Distribution of fumonisins in maize ears infected with strains of *Fusarium moniliforme* that differ in fumonisin production. Plant Disease 82, 953–958.
- Desjardins, A.E., Manandhar, G., Plattner, R.D., Maragos, C.M., Shrestha, K., McCormick, S.P., 2000. Occurrence of Fusarium species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. Journal of Agricultural and Food Chemistry 48, 1377–1383.
- Dombrink-Kurtzman, M.A., Dvorak, T.J., Barron, M.E., Rooney, L.W., 2000. Effect of nixtamalization (alkaline cooking) on fumonisin-contaminated corn for production of masa and tortillas. Journal of Agricultural and Food Chemistry 48, 5781-5786.
- Gong, Y.Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J., Wild, C.P., 2002. Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. British Medical Journal 325, 20–21.
- Harrison, L.R., Colvin, B.M., Greene, J.T., Newman, L.E., Cole, J.R., 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of *Fusarium moniliforme*. Journal of Veterinary Diagnostic Investigation 2, 217–221
- Hounhouigan, D.J., 1994. Fermentation of maize (*Zea mays* L.) meal for mawe production in Benin: physical, chemical and microbiological aspects. PhD Thesis, Agricultural University Wageningen, p. 99.
- Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H., Rombouts, F.M., 1993. Composition and microbiological and physical attributes of mawe, a fermented maize dough from Benin. International Journal of Food Science & Technology 28, 513-517.
- Hounhouigan, D.J., Massenon, A., Nago, C.M., Mestres, C., 1999. Caractéristiques physico-chimiques et aptitudes agronomiques de quelques variétés de maïs produites au Bénin. Annales des Sciences Agronomiques du Bénin 2, 115–128.
- Howard, P.C., Churchwell, M.I., Couch, L.H., Marques, M.M., Doerge, D.R., 1998. Formation of *n*-(carboxymethyl) fumonisin B₁, following the reaction of fumonisin B₁ with reducing sugars. Journal of Agricultural and Food Chemistry 46, 3546–3557.
- JECFA, 1998. Safety evaluation of certain food additives and contaminants. The Forty-Ninth Meeting of the Joint FAO/WHO

- Expert Committee on Food Additives (JECFA), World Health Organisation Food Additives Series, vol. 40, Geneva.
- Katta, S.K., Cagampang, A.E., Jackson, L.S., Bullerman, L.B., 1997. Distribution of *Fusarium* molds and fumonisins in drymilled corn fractions. Cereal Chemistry 74, 858–863.
- Keller, S.E., Sullivan, T.M., Chirtel, S., 1997. Factors affecting the growth of *Fusarium proliferatum* and the production of fumonisin B₁: oxygen and pH. Journal of Industrial Microbiology and Biotechnology 19, 305–309.
- Kellerman, T.S., Marasas, W.F.O., Thiel, P.G., Gelderblom, W.C.A., Cawood, M.E., Coetzer, J.A.W., 1990. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B₁. Onderstepoort Journal of Veterinary Research 57, 269–275.
- Kim, E.K., Scott, P.M., Lau, B.P.Y., Lewis, D.A., 2002. Extraction of fumonisins B₁ and B₂ from white rice flour and their stability in white rice flour, cornstarch, cornmeal, and glucose. Journal of Agricultural and Food Chemistry 50, 3614–3620.
- Kpodo, K., Sorensen, A.K., Jakobsen, M., 1996. The occurrence of mycotoxins in fermented maize products. Food Chemistry 56, 147–153
- Kpodo, K., Thrane, U., Hald, B., 2000. Fusaria and fumonisins in maize from Ghana and their co-occurrence with aflatoxins. International Journal of Food Microbiology 61, 147–157.
- Lopez-Garcia, R., Park, D.L., 1998. Effectiveness of postharvest procedures in management of mycotoxin hazards. In: Sinha, K.K., Bhatnagar, D. (Eds.), Mycotoxins in Agriculture and Food Safety, Eastern Hemisphere Distribution. Marcel Dekker, New York, pp. 407–433.
- Nago, C.M., 1997. La transformation alimentaire traditionnelle du maïs au Bénin : détermination des caractéristiques physicochimiques des variétés en usage. Relation avec l'obtention et la qualité des principaux produits dérivés. Thèse de Doctorat d'Etat. Université de Paris 7.
- Nago, M., Akissoe, N., Matencio, F., Mestres, C., 1997. End use quality of some African corn kernels: 1. Physicochemical characteristics of kernels and their relationship with the quality of "Lifin", a traditional whole dry-milled maize flour from Benin. Journal of Agricultural and Food Chemistry 45, 555–564.
- Nago, M., Hounhouigan, J.D., Akissoe, N., Zanou, E., Mestres, C., 1998. Characterization of the Beninese traditional ogi, a fermented maize slurry: physicochemical and microbiological aspects. International Journal of Food Science & Technology 33, 307–315.
- Pittet, A., 1998. Natural occurrence of mycotoxins in foods and feeds—an updated review. Revue Médicale Vétérinaire 149, 479–492.
- Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S., Van Schalkwyk, D.J., 1992. Fusarium moniliforme and fumonisins in corn in relation to human oesophageal cancer in Transkei. Phytopathology 82, 353–357.
- Schulthess, F., Cardwell, K.F., Gounou, S., 2002. The effect of endophytic *Fusarium verticillioides* on infestation of two maize varieties by lepidopterious stemborers and coleopteran grain feeders. Phytopathology 92, 120–128.
- Seefelder, W., Hartl, M., Humpf, H.U., 2001. Determination of *N*-(carboxymethyl) fumonisin B₁ in corn products by liquid

- chromatography/electrospray ionization-mass spectrometry. Journal of Agricultural and Food Chemistry 49, 2146–2151.
- Shephard, G.S., Leggott, N.L., Stockenstrom, S., Somdyala, N.I.M., Marasas, W.F.O., 2002. Preparation of South African maize porridge: effect on fumonisin mycotoxin levels. South African Journal of Science 98, 393–396.
- Shetty, P.H., Bhat, R.V., 1999. A physical method for segregation of fumonisin-contaminated maize. Food Chemistry 66, 371–374.
- Sinha, K.K., 1998. Detoxification of mycotoxins and food safety. In: Sinha, K.K., Bhatnagar, D. (Eds.), Mycotoxins in Agriculture and Food Safety, Eastern Hemisphere Distribution. Marcel Dekker, New York, pp. 381–405.
- Sydenham, E.W., Van Der Westhuizen, L., Stockenstrom, S., Shephard, G.S., Thiel, P.G., 1994. Fumonisin contaminated maize: physical treatment for the decontamination of bulk shipments. Food Additives and Contaminants 11, 25–32.
- Sydenham, E.W., Stockenstrom, S., Thiel, P.G., Shephard, G.S., Koch, K.R., Marasas, W.F.O., 1995. Potential of alkaline hydrolysis for the removal of fumonisins from contaminated corn. Journal of Agricultural and Food Chemistry 43, 1198–1201.
- Thiel, P.G., Sydenham, E.W., Shephard, G.S., 1996. The reliability and significance of analytical data on the natural occurrence of

- fumonisins in food. In: Jackson, L.S., DeVries, J.W., Bullerman, L.B. (Eds.), Fumonisins in Food, Advances in Experimental Medicine and Biology. Plenum Press, New York.
- Ueno, Y., Iijima, K., Wang, S.D., Suguira, Y., Sekijima, M., Tanaka, T., Chen, C., Yu, S.Z., 1997. Fumonisins as a possible contributing risk factor for primary liver cancer: a 3 year study of corn harvested in Haimen, China by HPLC and ELISA. Food Chemistry and Toxicology 35, 1143–1150.
- VICAM Science Technology, 1998. FumoniTest™ Instruction Manual. VICAM, Watertown, p. 39.
- Voss, K.A., Bacon, C.W., Meredith, F.I., Norred, W.P., 1996. Comparative subchronic toxicity studies of nixtramlized and water-extracted *Fusarium moniliforme* culture material. Food Chemistry and Toxicology 34, 623–632.
- Voss, K.A., Poling, S.M., Meredith, F.I., Bacon, C.W., Saunders, D.S., 2001. Fate of fumonisins during the production of fried tortilla chips. Journal of Agricultural and Food Chemistry 49, 3120–3126
- Wild, C.P., Hall, A.J., 2000. Primary prevention of hepatocellular carcinoma in developing countries. Mutation Research 462, 381–383.
- Wogan, G.N., 1992. Aflatoxins as risk factors for hepatocellular carcinoma I humans. Cancer Research 52, 2114.