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THE EFFECT OF PROCESSING PRACTICES ON MYCOTOXIN REDUCTION IN MAIZE BASED PRODUCTS: EVIDENCE FROM LACTIC ACID FERMENTATION IN SOUTHWEST NIGERIA

By

Oluwatoyin Ademola, Lenis Saweda O. Liverpool-Tasie, Adewale Obadina, Nikita Saha Turna, Felicia Wu

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EXECUTIVE SUMMARY

Because aflatoxin, a naturally occurring mycotoxin (fungal toxin) in maize and nuts, is known to cause liver cancer in humans, strategies to reduce aflatoxin in food are critical. Although fumonisin, another mycotoxin in maize, has not been conclusively linked to any human diseases, it can cause multiple adverse effects in other animal species and has been implicated in neural tube defects and growth impairment in human children. In this study, we examined the impact of lactic acid fermentation – a food processing method that has been used for possibly millennia in human populations – to decrease levels of aflatoxin and fumonisin in maize products in Nigeria. Our study showed that the mean total aflatoxin levels in processed maize samples (after lactic acid fermentation) were lower but not significantly different from mean levels in the raw maize product. Furthermore, even after processing, the mean total aflatoxin level in the samples of the final processed product was higher than the maximum acceptable limit shared by Nigeria and the European Union of 4μg/kg. However, we find strong evidence that lactic acid fermentation significantly reduced the mean levels of total fumonisins. Thus, while lactic acid fermentation can improve the food safety profile of maize, other strategies such as low initial levels in maize grain are likely necessary to guarantee a safe final product.

Key words: Aflatoxin, fumonisin, lactic acid fermentation, maize, Nigeria

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INTRODUCTION

Dietary mycotoxins – toxins produced by fungi that colonize food crops – cause multiple adverse health effects, including cancer, in humans and animals that consume them (Wu et al. 2014). Aflatoxins and fumonisins are two major groups of foodborne mycotoxins of major concern, particularly in developing countries. Fungi of the genera Aspergillus and Fusarium that infect food crops, including maize, produce these particular toxins. Though introduced to the African continent in the 1500s, maize has become a staple food crop throughout Africa. It accounts for 30-50% of low-income household expenditures in East and Southern Africa (IITA, 2013). It is also an important crop in West Africa, with Nigeria being the largest maize producing nation on the continent (FAOSTAT, 2012). While maize serves as an important ingredient for a rapidly growing animal feed industry in the country, 78% of the crop cultivated in Nigeria is consumed by humans (USDA, 2012). Thus, mycotoxins in maize remain a key concern for Nigerian public health.

All across Africa, maize is consumed in many different forms; including on the cob (boiled or roasted), as wet or dry cereal, steamed, as pudding/porridge, or as maize gruel. A popular cereal produced from maize through fermentation in Nigeria is ogi. It is an affordable maize-based product consumed widely across the nation for breakfast. Ogi is a very important weaning food for infants and a convenient meal for young children and those convalescing from illness (Onyekwere et al. 1989). Because of the consumption of ogi by potentially vulnerable populations such as young children and the elderly or ill, it is important to consider the risk of mycotoxins in this food product.

Aflatoxin and fumonisin are two of the most prominent mycotoxins in maize and maize products. Aflatoxins are responsible for 25,000-155,000 liver cancer cases worldwide per year (Liu and Wu, 2010), while fumonisins have been associated with human esophageal cancer in rural areas in South Africa (Rheeder et al. 1992), although the evidence for the latter is weaker. There is also increasing evidence that exposure to some of these mycotoxins may cause adverse immune system effects and stunted growth in children (Gong et al. 2004; Jiang et al. 2005; Khlangwiset et al. 2011; Mahdavi et al. 2010; Shuaib et al. 2010; Turner et al. 2003, 2007; Williams et al. 2004; Chen et al. 2018a, 2018b). Consequently, mycotoxin reduction and eventual elimination in commodities such as ogi frequently consumed by households and children needs to be a research and policy priority.

Many common methods of food processing may reduce mycotoxins (Voss et al. 1996; Shetty and Bhat, 1999). Physical, chemical, enzymatic and microbial methods of food processing that have been shown to decrease mycotoxin levels include sieve-cleaning, flotation density sorting, heating and washing as well as sorting, milling and extrusion (Karlovsky et al. 2016). Processing through lactic acid fermentation (as is done with ogi) is also expected to significantly reduce levels of mycotoxins (Mokoena et al. 2006; Shetty and Jespersen, 2006; Oluwafemi and Da-Silva, 2009; Cho et al. 2010; Nyamete, 2013; Zhao et al. 2015 Okeke et al. 2015). However, there is limited rigorous analysis of this phenomenon in Nigeria. Adegoke et al. (1994), Oluwafemi and Da-Silva (2009) and Okeke et al. (2015) are the only studies found on this topic. In their analyses, Adegoke et al. (1994) and Oluwafemi and Da-Silva (2009) did not consider fumonisins but focused on just one aflatoxin, AFB1. Furthermore, Adegoke et al. (1994) used the thin layer chromatography method

(TLC) while Oluwafemi and Da-Silva (2009) quantified mycotoxin levels with the enzyme linked immunosorbent assay (ELISA). However, due to the complexity of food samples coupled with possible low concentrations at which mycotoxin contamination can occur, a highly sensitive, selective and reliable analytical method for mycotoxin quantification is required.

LCMS/MS is a more recent methodology (that meets these requirements) and was used in this study to quantify the levels of seven mycotoxins including the four common aflatoxins (Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2) reported to be present in agricultural produce. Okeke et al. (2015) is the only study in Nigeria where the authors have used LCMS/MS to explore the effect of lactic acid fermentation on mycotoxins reduction in Nigeria. However, that study is restricted to one location and the study explored the effect of processing on mycotoxins for laboratory processed ogi. Since ogi is often purchased in wet form from processors in wet markets, studying commercial processors is important to understand how safe this commercially produced food product is and how the levels and potential reduction of aflatoxins and fumonisins vary with processing practices. For example, higher levels of mycotoxin exposure occur where moldy, broken and damaged maize grains are used (Njumbe Ediage et al. 2013; Ezekiel et al. 2014) and the quality of the raw material used actually influences the safety of fermented food products (Steinkraus, 1983). Studies have also shown that processing practices (Sadiku, 2010); the processing environment and hygiene of the personnel performing the art of fermentation (Iwuoha and Eke, 1996) are also key determinants of the safety of fermented products. As far as the authors are aware, no studies have been conducted on commercially produced ogi sold in wet markets in Nigeria. Thus, this study hopes to fill the gap. It focuses on assessing the prevalence of four different aflatoxins and three fumonisins in maize grain and ogi obtained from commercial ogi processors in southwestern Nigeria. The study explores the extent to which fermentation reduces mycotoxin levels in this important staple food in Nigeria and how this varies with various processing practices. This study extends beyond one location to cover ogi processors in three different states.

MATERIALS AND METHODS

Study area

This study was conducted in three out of six southwestern states in Nigeria (see Figure 1). This region of the country was selected because it is a region of high maize demand (for human and animal food). Furthermore, the area largely depends on maize from northern Nigeria, where majority of the maize is produced. Thus, the long supply chain for maize to the southwest could render the region more susceptible to mycotoxin contamination. Three towns (Ibadan, Abeokuta and Ikeja) were selected (one in each state) due the presence of dense ogi commercial centers.

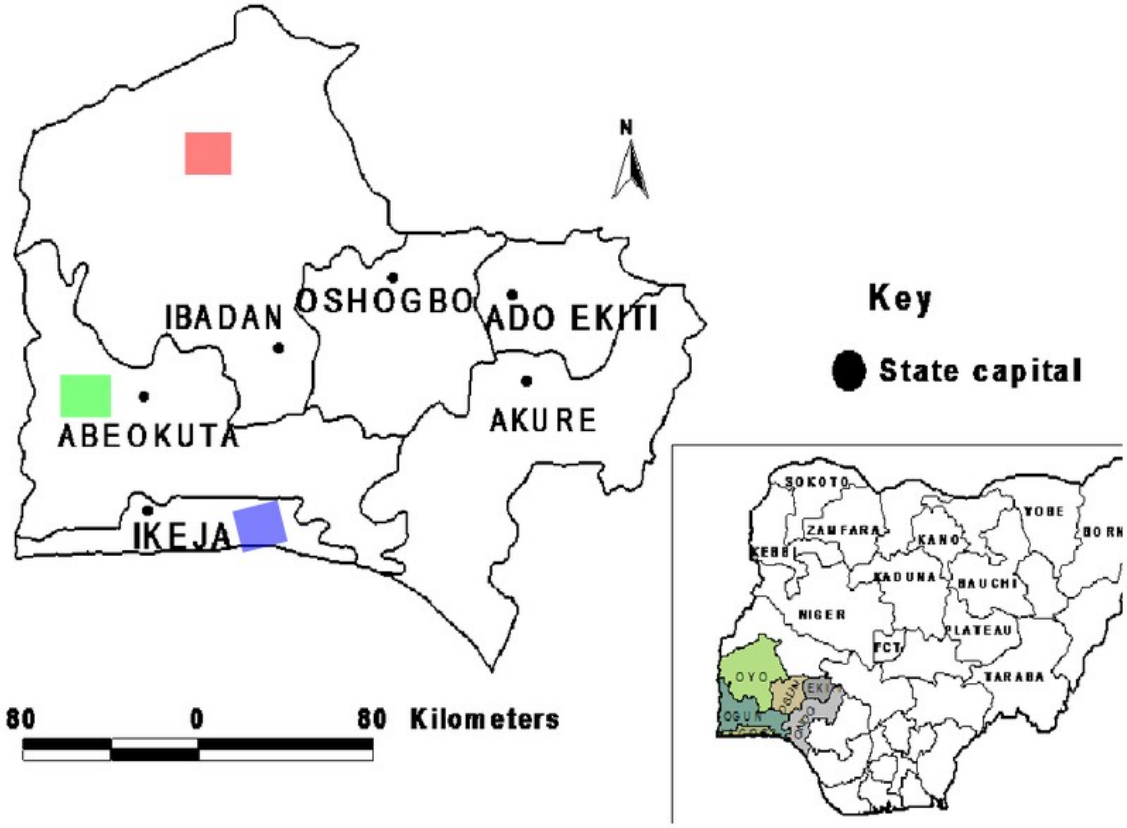

Figure 1. Map of South West Nigeria indicating the study locations. Source (inset map of Nigeria)

Note: The top left map shows the three study locations within their respective Nigerian state . Ikeja is the study location in Lagos State (purple box) while Abeokuta is the study location in Ogun State (green box) and Ibadan is the study location in Oyo State (red box) The bottom right map highlights the Nigerian states where the study locations (in the top left) are found. Oyo is light green, Ogun state is dark green and Lagos is depicted in brown. Source (inset map of Nigeria)

Sources of maize grain and ogi

Maize grain (raw material) and ogi (fermented maize final processed product) were obtained from ten randomly selected ogi processors in each of the three study locations . To understand how mycotoxin levels and reduction varied with processor practices, a structured questionnaire was administered to each processor to get information about their maize storage and processing practices (Appendix A). Five hundred grams (500 g) of maize grain were collected from each processor and milled. Fifty grams (50 g) from each milled sample was packed in a clean, properly labelled bag and transferred to the laboratory aseptically for mycotoxin analysis. Fifty grams (50 g) of the final product (ogi) was also purchased from each processor. The ogi was packed and labelled in a similar manner as the maize grain, transferred to the laboratory aseptically and both were stored at -20oC prior to mycotoxin analysis. Sixty (60) samples (30 maize and 30 ogi) were obtained from all the processors.

Commercial versus laboratory processing method of ogi

The general processing procedure for ogi production was similar across the three study locations. Maize grains were generally soaked in water and allowed to ferment (steeping) for 2-4 days (48-96 h). The softened grains were then washed, wet milled and sieved using a muslin cloth. The sieved paste was diluted with water in a container and left to ferment (souring) for 1- 2 days (24-48 h). The surface water was decanted and the sediment (wet paste) allowed to stand to sufficiently solidify. The solidified product was then measured into small units in clear polythene bags for sale. To distinguish potential practices that might affect mycotoxin reduction through ogi production, the practices of commercial processors is compared to the laboratory procedure articulated by Adebayo and Aderiye, (2007). The main differences between the laboratory processing of ogi and commercial processing is that there is a sorting stage before steeping in the lab processing that is not done by commercial processors (See Figure 2). In addition, the laboratory processing has no second fermentation (souring)

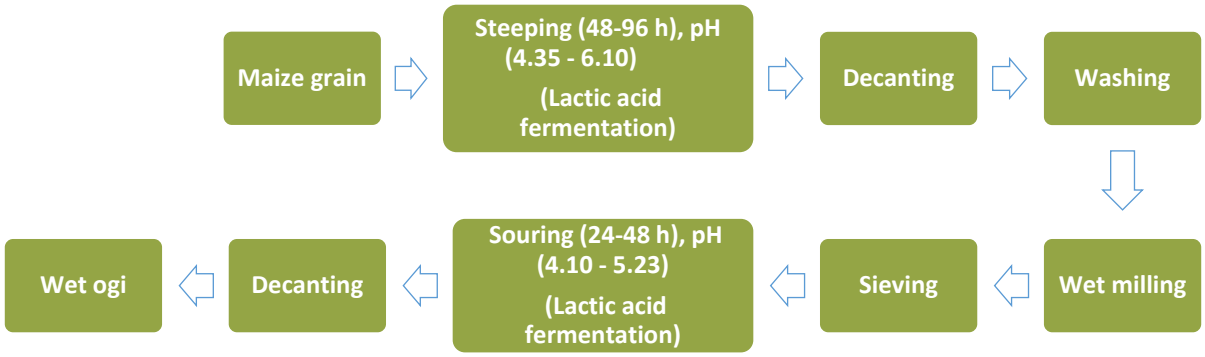

Figure 2. Flow chart of commercial and laboratory processing of ogi

Mycotoxin analysis of maize and ogi samples

Extraction of maize grains and ogi samples

The labeled maize and ogi samples were sent to Romer labs, USA, for mycotoxin analyses. Mycotoxin analysis of maize and ogi samples was performed by using liquid chromatography tandem mass spectrometry (LC-MS/MS). LC-MS/MS was used because of the low limit of detection of mycotoxins and multitoxins it can determine. The extraction of maize and ogi samples, apparent recoveries of analytes and detection of mycotoxin was carried out according to the method described by (Sulyok et al. 2007). Five grams of each sample was weighed into a 50 ml polypropylene tube and extracted with 20 ml of the extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). For spiking experiments, 0.25 g samples were used for extraction, Samples were extracted for 90 min on a GFL 3017 rotary shaker and diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v). 40 µl of the diluted extracts were injected into the LC instrument. Apparent recoveries of the analytes were crosschecked by spiking a sample that was not contaminated with mycotoxins with a multi-analyte standard on one concentration level. The spiked sample was stored overnight at ambient temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and the sample. The corresponding peak areas of the spiked samples were then used for the estimation of apparent recoveries by comparison to a standard prepared and diluted in neat solvent. All concentrations of the naturally contaminated samples were corrected by a factor equivalent to the reciprocal of apparent recovery (1/R; where R is the apparent recovery value) of each analyte.

LC-MS/MS parameters

Mycotoxins (mainly aflatoxins and fumonisins) were screened using a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo V electrospray ionization (ESI) source and a 1290 Series UHPLC System (Agilent Technologies, Waldbronn, Germany). Chromatographic separation was performed at 25◦C on a Gemini R _ C18-column, 150mm × 4.6 mmi d., 5 µm particle size, equipped with a C18 security guard cartridge, 4 mm × 3 mmi. d. (all from Phenomenex, Torrance, CA, USA). Positive analyte identification was confirmed by the acquisition of two MS/MS transitions, which yielded 4.0 identification points according to commission decision 2002/657/EC..

Data Analysis

SPSS for Windows v. 16.0 was used for the data analyses. Descriptive statistics and Two sample T-Tests tests were used to explore the occurrence and concentration of aflatoxins and fumonisins in maize and ogi obtained across the three study locations. Next, the study explored mycotoxin reduction levels in freshly fermented ogi and how this varied with processing practices. The Duncan's Multiple Range test (DMRT) was used to separate the means at $p<0.05$ significance level by one-way analysis of variance. The Two sample T- test (between subject T-test) was also used to compare mycotoxin reduction levels across different groups of processors depending on how long their maize grain had been stored and how long they steeped their maize during processing.

Results and discussions

Characteristics of the ogi processors

The procurement and storage practices of the study processors across the three locations are presented in Table 1. Seventy three percent (73 %) of the processors stored their maize for less than 7 days while 27 % stored maize for more than 7 days. Almost 50 % of processors did not store their maize before processing. This is because they typically buy small quantities from the market; just enough to produce their desired quantity of ogi. For those who did store, the most common storage method used across the three locations was a plastic container; used by 63 % of processors. The plastic containers are made from hard plastic and typically uncovered. Thus, exposure to moisture and heat is likely to be high. Thirty two percent (32 %) and seven (7 %) used a jute bag and polythene bag respectively. The majority of the processors (90 %) claimed not to have problems with insects/rats/mold infestation and 67 % usually clean their storage structures before use.

During the process of ogi production, no processors sorted their maize before steeping . Forty percent (40 %) steeped their maize for 2 days, fifty-seven (57 %) for three days and three (3 %) steeped for four days. While most processors in Ibadan and Abeokuta steeped for two days, 70 % of processors in Lagos steeped for three days. Most processors (97 %) allowed their maize to undergo souring for one day while only one (3 %) processor soured for 2 days.

Table 1: Storage and processing characteristics of the ogi processors

Parameters	Number observed (%)			
	Total	**Abeokuta**	**Ibadan**	**Lagos**
Length of storage				
< 7 days	22(73)	8(80)	7(70)	7(70)
>7 days	8(27)	2(20)	3(30)	3(30)
Storage structure				
Plastic container*	10(63)	2(50)	4(64)	4(64)
Jute sack on cemented floor*	5(31)	1(25)	2(36)	2(36)
Polythene bag*	1(7)	1(25)	None	None
None	14(47)	6(60)	4(40)	4(40)
Location of purchase of maize				
South	30(100)	10(100)	10(100)	10(100)
North	None	None	None	None

Problem with insect/rat/mold				
Yes	3(10)	None	2(20)	1(10)
No	27(90)	10(100)	8(80)	9(90)
Cleaning of storage structure before use				
Yes	10(33)	2(20)	4(40)	4(40)
No	20(67)	8(80)	6(60)	6(60)
Sorting of maize before processing				
Yes	None	None	None	None
No	30(100)	10(100)	10(100)	10(100)
Number of days of steeping/soaking				
2	12(40)	3(30)	2(20)	7(70)
3	17(57)	7(70)	7(70)	3(30)
4	1(3)	None	1(10)	None
Number of days of souring				
1	29(97)	10(100)	9(90)	10(100)
2	1(3)	None	1(10)	None

Source: Authors calculation Note: * means conditional on storing

Occurrence of aflatoxins and fumonisins in maize grain and ogi

Seven mycotoxins - aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), fumonisin B1(FB1), fumonisin B2 (FB2), and fumonisin B3 (FB3) - were quantified in all samples. Mean total aflatoxin and fumonisin levels before and after fermentation are shown in Figures 3 and 4. The limit of detection (LOD) for aflatoxins ranged from 1.1 to 1.6 μg/kg while the LOD for fumonisins was 100 μg/kg. Maize samples obtained from Ibadan and Abeokuta tended to have higher levels of mycotoxins than Lagos. The mean total aflatoxin level in maize samples from Ibadan before fermentation was 9.10 μg/kg while the mean total aflatoxin level in the "ogi" samples (after fermentation) was 5.55 μg/kg. In Abeokuta, the mean total aflatoxin level in maize samples before fermentation was 18.35 μg/kg (more than double that of Ibadan and 5 times the maximum acceptable limit recommended by European Union of 4 μg/kg). After fermentation, the mean total aflatoxin level was 5.62 μg/kg. Maize samples from Lagos had total aflatoxin levels less than LOD. This may mean that ogi processors from Lagos were able to purchase good quality maize grain and/or prevent contamination of their purchased maize during storage. A t-test comparing the mean total aflatoxin levels before and after processing revealed that though the mean levels after fermentation were lower, they were not significantly different from the mean levels of the raw maize samples prior to fermentation.

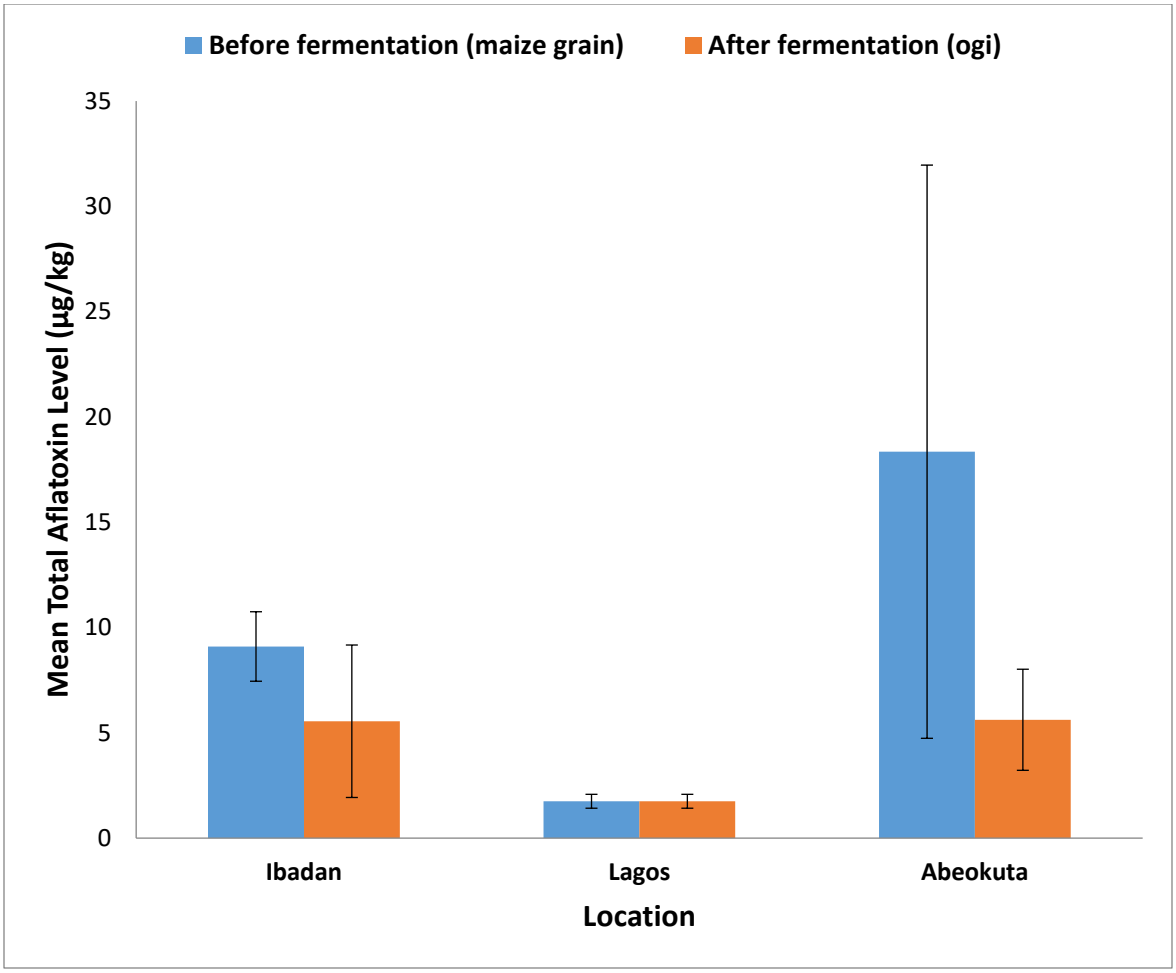

Figure 3. Mean Total aflatoxin levels of maize and fermented ogi sold in southwestern Nigeria. Note: Total aflatoxin refers to the sum of AFB1, AFB2, AFG1 and AFG2. Vertical lines on bars indicate the standard error of mean

For fumonisins, prior to fermentation, the mean total levels in maize samples were (495, 185.50 and 335 µg/kg) for Ibadan, Lagos and Abeokuta respectively. After processing, the levels of total fumonisin in the fermented product (ogi) for Lagos and Abeokuta were less than LOD and 187.50 µg/kg in Ibadan, which is much lower than the levels in the raw material (maize grain). A t-test comparing the mean levels of total fumonisin before and after fermentation indicate that the mean differences were statistically significant in all three study locations. These results indicate that processing maize through lactic acid fermentation significantly lowers the levels fumonisins. This result is consistent with Okeke et al. (2015), though that study also found a significant reduction in mean aflatoxin levels which we do not find. While Fandohan et al. (2005) like Okeke et al. (2015) found a significant reduction in mean aflatoxin levels in maize gruel in the Republic of Benin, they did not find a significant reduction in fumonisin levels. Contrary to both studies, these results suggest that fermented ogi might still not be completely safe for consumption. The mean total aflatoxin level in the fermented product in the two study locations where the raw maize product had mean levels higher than LOD was still higher than the maximum acceptable limit in Nigeria (also recommended by European Union) of 4 µg/kg total aflatoxin (AFB1+AFB2+AFG1+AFG2) (EU, 2006). This suggests that while a significant reduction is possible, lactic acid fermentation might not eliminate aflatoxins to safe levels in foods after processing. Thus local foods such as fermented ogi are not completely safe for consumption and

remain a public health concern since ingesting even low concentration of these toxins in food over time may predispose consumers to infection and disease.

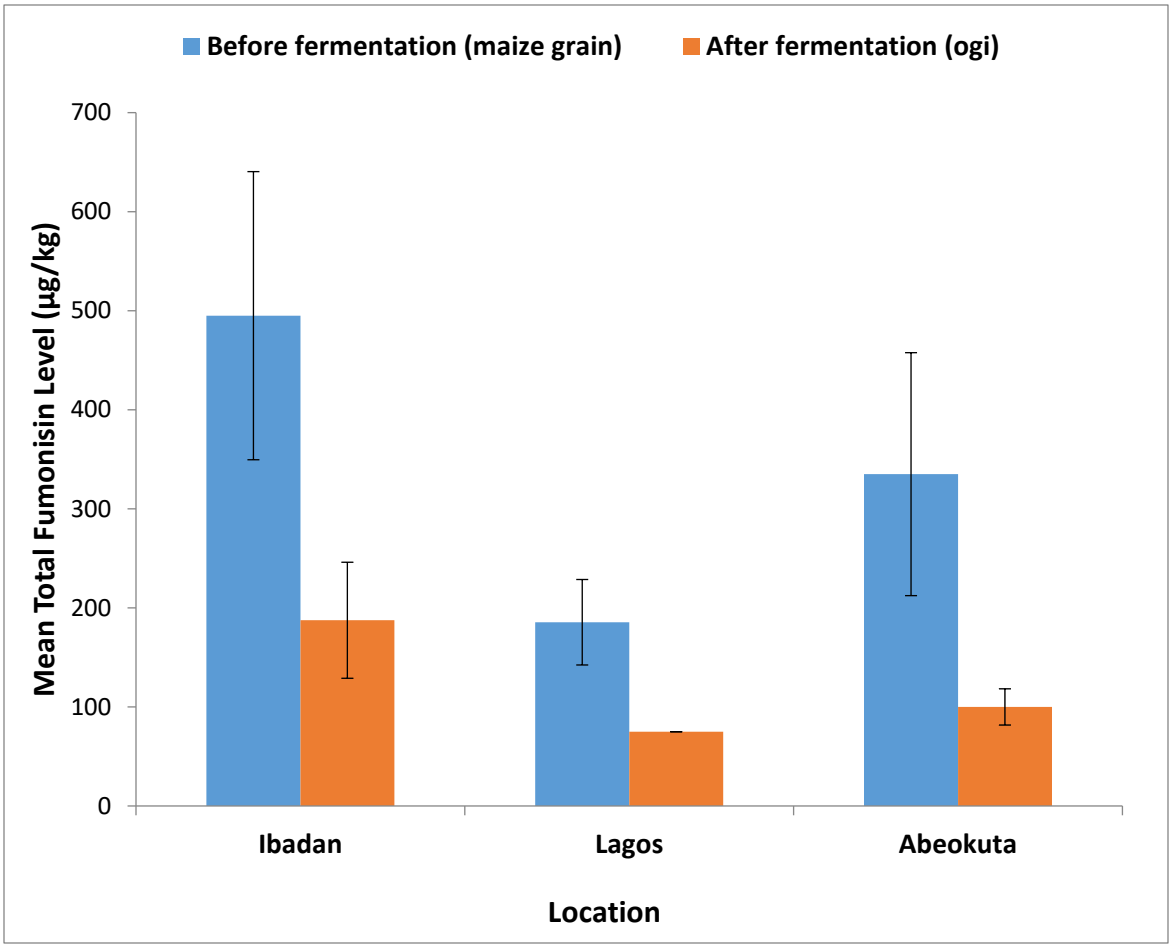

Figure 4. Mean Total fumonisin levels of maize and fermented ogi sold in southwestern Nigeria Note: Total fumonisin refers to the sum of FB1, FB2 and FB3. Vertical lines on bars indicate the standard error of mean

Magnitude of mycotoxin reduction due to lactic acid fermentation

The percentage reduction of fumonisins in maize due to lactic acid fermentation across the three locations is displayed in Table 2. Since our mean total aflatoxin levels before and after fermentation were not significantly different, we focus on the reduction levels for fumonisins[1]. Estimates were based on percentage differences between fumonisin levels in the maize grain and final product - ogi. For total fumonisins high and significant levels of percentage reduction in maize grain from fermentation was observed in all the study locations. These were 62.12 %, 58.90 % and 70.15 % (Ibadan, Lagos and Abeokuta respectively). This confirms that fermentation of maize influenced by lactic acid bacteria is associated with significant reductions in fumonisins in South West Nigeria. This finding is consistent with Okeke et al. (2015) who reported approximately 85 % reduction in fumonisins in white and yellow maize grain for ogi production in Ogun state Nigeria. However, it

[1]Not surprising, the highest percentage reduction in mean total aflatoxin levels (69.37 %) after fermentation was observed in Abeokuta with the highest pre fermentation aflatoxin levels.

contrasts with the findings of Fandohan et al. (2005) who reported small (nd statistically insignificant) effects of lactic acid fermentation on fumonisin levels (13 %) in the Republic of Benin. As mentioned earlier, though fermentation reduces mycotoxins, the final levels of mycotoxin in the final product (fermented ogi) will also depend on the initial level of mycotoxin in the raw material (maize). Thus, it is important to ensure proper pre and postharvest practices of maize grain (storage and handling) to guarantee a safe final product. Though the findings of this study are consistent with those of Okeke et al. (2015), the reduction levels for the different mycotoxins found in this study are consistently lower than theirs. This might be due to external factors and processing practices adopted by processors not accounted for in a laboratory setting and reflects the importance of conducting a study with actual processors.

Table 2: Reduction (%) of mycotoxins in fermented *ogi* due to fermentation of maize grain

Location	Initial level of Total FBs in maize (µ/kg)	(%) Mycotoxin Reduction of Total FBs
Ibadan	495	62.12
Lagos	182.5	58.90
Abeokuta	335	70.15

Source: Authors calculation. Note: FBs refers to Fumonisins

Effect of storage and processing practices on aflatoxin and fumonisins concentration

The effect of the length of steeping on the reduction of aflatoxins and fumonisins through lactic acid fermentation.
Steeping is an important process of maize grain fermentation via soaking prior to milling and also because it releases germs, which allows for the breakdown of protein matrix (Karlovsky et al. 2016). Water-soluble toxins migrate from grains to steep water, which facilitates mycotoxin reduction (Canela et al. 1996). Steeping time among the study processors ranged between two and four days. Table 3 displays the mean reduction of aflatoxins and fumonisins level in ogi due to steeping across the three locations . There is no statistically significant difference in the mean level of aflatoxin reduction due to the length of steeping in Ibadan and Lagos. It is only in Abeokuta, that there is a statistically significantly different mean reduction for (AFG1) those who steeped for two days compared to those who steeped for three. The lack of significant differences in mean reduction for different lengths of steeping in the other locations might be due to the limited variation in the number of days of steeping and indicates that the general steeping practices of processors do not significantly affect the effectiveness of lactic acid fermentation. Other studies have found that extended fermentation could increase acidic conditions, which interfere with mycotoxin reduction Kpodo et al. (1996) and Okeke et al. (2015). Thus, caution on unnecessarily extending fermentation days is important.

As mentioned earlier, most of the maize grain obtained in Lagos for ogi production had levels of mycotoxins that were less than limit of detection except for FB1 and FB3. Thus, it is not surprising that there is no variation in these mycotoxins due to steeping. For FB1 and FB3, the levels of reduction do not systematically differ by length of steeping.

The effect of length of maize storage on the reduction of aflatoxins and fumonisins through lactic acid fermentation

Table 4 shows the effect of length of maize grain storage on the level of reduction of aflatoxins and fumonisins concentration due to processing (lactic acid fermentation) across the three study locations in southwestern Nigeria. The length of maize storage in our sample ranged from 0-14 days and varied across locations. The average number of days that maize was stored by ogi processors was seven in Ibadan and Abeokuta while it was eight in Lagos. Processors were divided into two groups based on how long they stored their maize grain before processing. The first group consisted of those who stored maize grain for less than seven days before processing while the second groups are those who stored for more than seven days. There is typically no significant difference in mean reduction levels of the different aflatoxins for the two groups of processors (storing less than or greater than 1 week) in all study locations. These results might be driven by the generally low storage periods of the maize (typically less than 2 weeks). For fumonisins, statistically significant and comparable, higher levels of reduction of fumonisins were observed for samples stored longer than a week. Since length of maize storage is not typically associated with growth in fumonisins, this likely indicates the presence of other environmental factors or unobserved processor practices that are correlated with their length of maize storage but which affect the reduction of fumonisins.

The effect of storage structure on the reduction of aflatoxins and fumonisins through lactic acid fermentation.

The three storage methods used by processors include storage in plastic containers (a hard plastic container without a cover), storage in jute sacks on cemented floors (typically well covered and on a relatively cool surface) and polythene plastic bags (soft plastic bags similar to shopping bags in a typical grocery store). Processors in Ibadan and Lagos stored their maize in plastic containers or jute sacks on cemented floor prior to use while processors in Abeokuta store their maize in either plastic containers, jute sack on cemented floor or in polythene bags. Some processors stored their maize grain for less than a day and their storage structure was categorized as none. The reduction in the level of aflatoxins and fumonisins in samples obtained from Ibadan and Lagos were generally not significantly different ($P>0.05$) for the different storage structures (see Table 5). However, in Abeokuta, while the level of reduction in AFB1, AFB2 and AFG1 for the different storage structures were not significantly different ($P>0.05$), there is a statistically significantly higher fumonisin reduction forFB1 and FB2 for ogi produced with maize grain stored in jute sack and plastic container compared to those in polythene bag. The highest level of reduction occurs with the Jute sack, which seems consistent with the fact that such storage structure had the maize well covered and stored on a relatively cool and clean surface.

Table 3. Mean reduction of aflatoxins and fumonisins level in *ogi* due to steeping length

Location	Duration of steeping (days)	Level of aflatoxin reduction (µg/kg)				Level of fumonisin reduction (µg/kg)		
		AFB_1	AFB_2	AFG_1	AFG_2	FB_1	FB_2	FB_3
Ibadan	2	6.20 ± 3.39^{a}	<LOD	3.15 ± 1.91^{a}	<LOD	537.50 ± 512.65^{b}	50.00 ± 70.710^{a}	37.50 ± 53.03^{a}
	3	4.88 ± 2.84^{a}	<LOD	2.73 ± 1.81^{a}	<LOD	345.83 ± 118.76^{ab}	41.67 ± 71.880^{a}	<LOD
	4	6.10 ± 0.00^{a}	<LOD	3.30 ± 0.00^{a}	<LOD	<LOD	175.00 ± 0.00^{a}	<LOD
Lagos	2	<LOD	<LOD	<LOD	<LOD	89.29±116.24	<LOD	10.71±28.35
	3	<LOD	<LOD	<LOD	<LOD	150.00±198.43	<LOD	<LOD
Abeokuta	2	5.00±1.93	<LOD	1.30±2.25*	<LOD	33.33±226.84	<LOD	<LOD
	3	17.99±44.96	3.01±6.82	0.13±0.87	<LOD	246.43±315.38	75.00±109.92	<LOD

Source: Authors calculation. Note: Mean with the same lowercase letters in a row are not significantly different ($p>0.05$). For steeping length in Lagos and Abeokuta where there are only have two groups (2 vs 3), the study used the two sample T-test to test for significant difference in sample means.* indicates means are statistically significantly different at 5%

Table 4. The effect of length of maize storage on aflatoxins and fumonisins reduction

Location	Length of storage (days)	Level of aflatoxin reduction (μg/kg)				Level of fumonisin reduction (μg/kg)		
		AFB_1	AFB_2	AFG_1	AFG_2	FB_1	FB_2	FB_3
Ibadan	0-6	2.31±9.62	<LOD	0.71±7.11	<LOD	314.29±313.87	64.29±94.49*	10.71±28.35*
	7-14	4.30±2.29	<LOD	2.00±1.01	<LOD	316.67±150.69	<LOD	<LOD
Lagos	0-6	<LOD	<LOD	<LOD	<LOD	117.86±157.93*	<LOD	<LOD
	7-14	<LOD	<LOD	<LOD	<LOD	83.33±87.80*	<LOD	<LOD
Abeokuta	0-6	15.41±41.90	2.53±6.45	0.49±1.38	<LOD	137.50±182.25*	9.38±26.52*	<LOD
	7-14	6.10±12.45	0.45±0.64	0.40±2.12	<LOD	687.50±123.74*	225.00±123.74*	<LOD

Source: Author's calculation. Note:*significant at ($p<0.05$)

Table 5. The effect of storage structure on the reduction of aflatoxins and fumonisins through lactic acid fermentation.

Location	Storage structure	Level of aflatoxin reduction (μg/kg)				Level of fumonisin reduction (μg/kg)		
		AFB_1	AFB_2	AFG_1	AFG_2	FB_1	FB_2	FB_3
Ibadan	None	0.14 ± 10.56^{a}	<LOD	1.22 ± 7.77^{a}	<LOD	210.00 ± 206.61^{a}	20.00 ± 92.53^{a}	<LOD
	Plastic container	6.50 ± 3.11^{a}	<LOD	3.82 ± 1.84^{a}	<LOD	481.25 ± 305.76^{a}	87.50 ± 72.17^{a}	18.75 ± 37.50^{a}
	Jute sack	3.80 ± 0.00^{a}	<LOD	1.80 ± 0.00^{a}	<LOD	175.00 ± 0.00^{a}	<LOD	<LOD
Lagos	None	<LOD	<LOD	<LOD	<LOD	110.00 ± 166.40^{a}	<LOD	<LOD
	Plastic container	<LOD	<LOD	<LOD	<LOD	91.67 ± 158.77^{a}	<LOD	<LOD
	Jute sack	<LOD	<LOD	<LOD	<LOD	125.00 ± 70.71^{a}	<LOD	<LOD
Abeokuta	None	20.55 ± 48.28^{a}	3.37 ± 7.40^{a}	0.65 ± 1.59^{a}	<LOD	45.83 ± 157.65^{a}	<LOD	<LOD
	Plastic container	7.45 ± 10.54^{a}	0.451 ± 0.64^{a}	0.55 ± 0.78^{a}	<LOD	387.50 ± 548.01^{ab}	137.50 ± 194.45^{ab}	<LOD
	Jute sack	2.70 ± 0.00^{a}	<LOD	1.90 ± 0.00^{a}	<LOD	600.00 ± 0.00^{b}	175.00 ± 0.00^{b}	<LOD
	Polythene bag	<LOD	<LOD	<LOD	<LOD	175.00 ± 0.00^{ab}	75.00 ± 0.00^{ab}	<LOD

Source: Author's calculation *Note: Mean with the same lowercase letters in a row are not significantly different (p>0.05)*

DISCUSSIONS

Lower mean levels of total aflatoxin were found in processed maize after lactic acid fermentation but these were not statistically significantly different from mean levels of the raw maize product. Furthermore mean total aflatoxin levels after fermentation (where initial levels were higher than LOD) were still higher than levels considered safe for consumption. The mean level of total fumonisins in all three study locations was generally below maximum acceptable limits of 1000 μg/kg set by the European Union (EU, 2006) for maize grain2. However, this study finds even lower levels of fumonisins in ogi samples. This suggests that lactic acid fermentation is still able to significantly reduce the levels of this toxin and this is an important finding not widely documented in the literature.

Consistently, lower levels of fumonisin reduction was found among actual food processors than has been found in laboratory settings. This confirms the importance of exploring the effects of strategies to reduce mycotoxins (such as processing) in non-laboratory environments that are more likely to reflect reality.

While lactic acid fermentation is generally effective for fumonisin reduction, the study finds some evidence that processor practices affect the effectiveness of lactic acid fermentation. Where significant, higher levels of aflatoxin reduction were recorded for ogi produced from maize that had been steeped for two days, compared to three or four days. Thus, fermentation should not be unnecessarily extended to allow the growth of additional molds. Similarly, this study finds small effects of storage structure on the effectiveness of fumonisin reduction. Significant higher levels of fumonisin reduction was found among processors who store in jute bags. These bags are generally perceived to be the best storage structure to prevent maize grain exposure to moisture, as they are well covered and placed on a cement floor.

CONCLUSIONS

Maize is an important staple food crop consumed all across Africa. In many parts of the continent, it is used for "ogi", a weaning food for babies or a meal for the convalescing. Ogi is produced through lactic acid fermentation of maize. This study explored the extent to which lactic acid fermentation of maize could reduce the level of mycotoxins (aflatoxins and fumonisins) in maize based products. The study also explored how mycotoxin reduction varies with storage and processing practices.

Samples of both maize (raw material) and ogi (final product) were collected from commercial processors in three urban regions in southwest Nigeria, and analyzed for aflatoxin and fumonisin. While lower mean total aflatoxin levels were found in maize after lactic acid fermentation, these means were not statistically significantly different from the mean of the samples prior to fermentation. For total fumonisins, high percentage reductions in maize grain from fermentation were observed in all the study locations: 62.12 %, 58.90 %, and 70.15 % fumonisin reductions in maize samples from Ibadan, Lagos, and Abeokuta, respectively.

Even after processing, the mean total aflatoxin level in the ogi samples (in all locations where initial levels where higher than LOD) was slightly higher than the maximum acceptable limit shared by Nigeria and the European Union of 4 μg/kg. Thus, while lactic acid fermentation can improve the food safety profile of maize, other strategies such as low initial levels in maize grain

[2] Currently, there is no regulation on fumonisin levels in food products in Nigeria

are likely necessary to guarantee a product that meets this strict aflatoxin standard. Proper storage and processing practices can also play a role in improving the safety of maize processed through lactic acid fermentation.

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APPENDIX 1

Questionnaire on practices during processing/ *ogi* production

Michigan State University (MSU)
Feed the Future Food security Policy/Nigeria Agricultural Policy Project

"We are part of a team at Michigan State University, who are studying aspects to do with agricultural development in Nigeria. Your participation in answering these questions is very much appreciated. Your responses will be **COMPLETELY CONFIDENTIAL**.. If you indicate your voluntary consent by participating in this interview, may we begin? If you have any questions or comments about this survey, you may contact Dr. Saweda Tasie, Assistant Professor, International Development, Department of Agricultural, Food, and Resource Economics, Justin S. Morrill Hall of Agriculture 446 West Circle Drive, Room 219, Michigan State University; **Tel:**; email: lliverp@msu.edu"

LGA:
Name of processor:

1. Where do you get your maize from?
   Northern part of Nigeria
   Southern part of Nigeria
2. Where in the North or South do you get your maize from?
3. How long do you normally store before selling or processing (days)
4. How long did you store this maize? (days)
5. What storage method do you use?
   - In jute sacks on a raised platform
   - In jute sacks on bare floor
   - In jute sacks on cemented floor
   - Cribs
   - On the roof
   - In a rhombus
   - Straw hut
   - Others
6. Why do you store maize this way
7. Do you have problems with insects? Yes No
   How do you address this?
   - a. Apply chemicals
   - b. Apply pepper
   - c. Use air tight bags
   - d. Others…….. Specify
8. Do you have problems with mice/rats? Yes No
   How do you address this?
   - a. Apply chemicals
   - b. Apply pepper
   - c. Use air tight bags

d. Others…….. Specify

9. Do you have problems with fungi/mould? Yes No

How do you address this?

a. Apply chemicals
b. Apply pepper
c. Use air tight bags
d. Others…….. Specify

10. Do you have problems with maize Theft? Yes No

How do you address this?

11. Do you clean the storage structure before storage? Yes No

12. If you treat the storage structure before use, what method did you use?

Ash+pepper
Fumigation (specify)
Local leaves
Pesticides (specify)
Others

13. Are the treatments that you use successful?

14. How did you confirm this? Yes No

15. Do you normally sort your maize before use Yes No

16. How long (days) do you normally soak

One
Two
Three
Four
Others

17. How long (days) do you normally ferment

One
Two
Three
Four
Others