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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

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EXECUTIVE SUMMARY

Aflatoxin, a naturally occurring mycotoxin in maize and nuts, is known to cause liver cancer in humans; hence, strategies to reduce aflatoxin in food are critical. Although fumonisin, another mycotoxin in maize, has not been conclusively linked to any human diseases, it causes multiple adverse effects in other animal species and has been implicated in neural tube defects and growth impairment in human infants and children. This study examined the impact of lactic acid fermentation – a food and beverage processing method that has been used for millennia in human populations – to decrease levels of aflatoxin and fumonisin in maize products in Nigeria. We found that some aflatoxin levels were significantly reduced by lactic acid fermentation. However, even after processing, the mean total aflatoxin level in samples of the final product was typically close to the maximum acceptable limit shared by Nigeria and the European Union 4μg/kg. On the other hand, lactic acid fermentation significantly reduced the levels of fumonisin. Thus, while lactic acid fermentation can improve the food safety profile of maize, other strategies such as low initial levels in maize grain and other mycotoxin control methods are likely necessary to guarantee a product that meets Nigerian/EU aflatoxin standards.

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

CONTENTS

INTRODUCTION

Mycotoxins – toxins produced by fungi that colonize food crops – cause multiple adverse health effects, including cancer, in humans and animals (Wu et al., 2014). Aflatoxins and fumonisins are two major groups of foodborne mycotoxins of major concern in developing countries. Fungi of the genera Aspergillus and Fusarium that infect food crops, including maize, produce these particular toxins. They are of particular concern in maize in tropical and subtropical world regions, because warm climates encourage the growth of these fungi. First 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 among the two largest maize producing nations on the continent (FAOSTAT, 2017). 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, the mycotoxins that naturally occur in maize are a concern for Nigerian public health.

All across Africa, maize is consumed in many different forms; including on the cob (boiled or roasted), wet or dry cereal, steamed custard, pudding, porridge, and 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 have been estimated to cause 25,000-155,000 liver cancer cases worldwide per year (Liu and Wu, 2010), while fumonisins have been associated with neural tube defects in infants whose mothers were exposed during pregnancy (Missmer et al., 2005). In the past, fumonisin exposure was also associated with increased risk of esophageal cancer, although the evidence is more limited (Rheeder et al., 1992). There is also increasing evidence that exposure to aflatoxin or fumonisin may compromise immunity and contribute to stunted growth in children (Chen, Mitchell, et al., 2018; Chen, Riley, et al., 2018; Gong et al., 2004; Jiang et al., 2005; Khlangwiset et al., 2011; Mahdavi et al., 2010; Shuaib et al., 2010; Turner et al., 2007; Williams et al., 2004). Consequently, mycotoxin reduction in commodities such as ogi frequently consumed by households and children should be a food safety priority.

Many common methods of food processing may reduce mycotoxin levels. Physical, chemical, enzymatic and microbial methods of food processing that have been shown to decrease mycotoxin levels include sieve-cleaning, flotation density sorting, baking, frying, roasting, sorting, milling and extrusion (Karlovsky et al., 2016; Kaushik, 2015; Voss et al., 2017). Processing through lactic acid fermentation (as is done with ogi) is also expected to significantly reduce levels of mycotoxins (Cho et al., 2010; Mokoena et al., 2006; Nyamete et al., 2016; Okeke et al., 2015; Oluwafemi and Da-Silva, 2009; Shetty and Jespersen, 2006; Zhao et al., 2015). However, there is limited rigorous

analysis of this phenomenon in foods processed in Nigeria. Adegoke et al. (1994), Oluwafemi and Da-Silva (2009), and Okeke et al. (2015). Adegoke et al. (1994) and Oluwafemi and Da-Silva (2009) did not examine changes in fumonisin levels, but focused on just one aflatoxin, AFB1 (the most toxic of the aflatoxins). 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 analyzing 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 (AFB1, AFB2, AFG1, and AFG2) reported to be present in agricultural produce. This study also considers the three fumonisins that have been reported in food: FB1, FB2, and FB3 (Shephard et al. 1996). 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 was restricted to one location, and the study explored the effects 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 (Ediage et al., 2013; Ezekiel et al., 2014). 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.

Thus far, no studies have been conducted on commercially produced ogi sold in wet markets in Nigeria. This study helps to fill that gap. We have assessed the prevalence of four aflatoxins and three fumonisins in maize grain and ogi obtained from commercial ogi processors in southwestern Nigeria. The study determines the extent to which fermentation reduces mycotoxin levels in this important staple food in Nigeria and covers ogi processors in three different states.

MATERIALS AND METHODS

Study area

This study was conducted in three southwestern states in Nigeria (see Figure 1). This region of the country was selected because it is a region of high maize demand for both human food and animal feed. Moreover, the area largely depends on maize from northern Nigeria, where the majority of the nation's maize is produced. Thus, the relatively long supply chain for maize to reach 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.

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. While a formal listing was not conducted in the commercial centers/markets, ogi processors were systematically selected across the different parts of the markets and times of operation within a day. To understand the factors that could affect how mycotoxin levels and their relative reduction varied with processor practices, a structured questionnaire was administered to each processor about their maize storage and processing practices (Appendix 1). 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 labeled 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 labeled in a similar manner as the maize grain, transferred to the laboratory aseptically and both were stored at -20oC prior to mycotoxin analysis. Sixty 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 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 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 were compared to the laboratory procedures described in Adebayo and Aderiye (2007). The main differences between the laboratory processing of ogi and commercial processing are that there was a sorting stage before steeping in the lab processing that is not done by commercial processors (See **Figure 2**); and the laboratory processing had no second fermentation step (souring), which ogi processing companies often employ.

Mycotoxin analysis of maize and ogi samples

Extraction of maize grains and ogi samples

The labeled maize and ogi samples were sent to RomerLabs, USA, for mycotoxin analyses. Mycotoxin analyses of maize and ogi samples were 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 mycotoxin analyses were 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 by volume). 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 by volume). 40 µl of the diluted extracts were injected into the LC instrument. Apparent recoveries of the analytes were cross-checked 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

Descriptive statistics were used to explore the occurrence and concentration of aflatoxins and fumonisins in maize and ogi obtained across the three study locations. The non-parametric Wilcoxon sign rank test of matched pairs was used to test for significant differences in mycotoxin levels before and after processing. Next, the study explored mycotoxin reduction levels in freshly fermented ogi and how this varied with processing practices. Processors were divided into groups depending on how long they stored their maize before processing and how long they steeped their maize during processing. To test the effect of these practices on mycotoxin reduction the non-parametric two-sample Wilcoxon rank-sum (Mann-Whitney) test was also used. A $p \leq 0.05$ was considered to be statistically significant for all the statistical tests.

RESULTS

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 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% reported cleaning their storage structures before use.

During the process of ogi production, no processors sorted their maize before steeping (soaking the maize grain for initial fermentation). 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 the maize for two days, 70% of processors in Lagos steeped for three days.

Most processors (97%) allowed their maize to undergo souring (soaking of the milled maize for additional fermentation) for one day while only one processor soured for 2 days.

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. The geometric mean of 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 1.6μg/kg while the LOD for fumonisins was 100μg/kg. Maize samples obtained from Ibadan and Abeokuta tended to have higher levels of mycotoxins than Lagos. The geometric mean total aflatoxin level in maize samples from Ibadan before fermentation was 9.06μg/kg while the geometric mean total aflatoxin level in the ogi samples (after fermentation) was 3.91μg/kg. In Abeokuta, the geometric mean total aflatoxin level in maize and ogi were 6.04μg/kg and 4.82μg/kg respectively. Maize samples from Lagos had geometric mean total aflatoxin consistently less than LOD.

We reject the null hypothesis that our data for both the maize and ogi samples are normal. Thus a non-parametric Wilcoxon sign rank test of matched pairs was used to compare the median aflatoxin levels before and after processing, it revealed that the levels of AFB1, AFG1, AFG2 and total aflatoxin after processing were significantly lower than the initial levels in the maize in Ibadan. However, the median level of the different aflatoxins studied were not statistically significantly different after processing in Lagos and Abeokuta (**Table 2**).

For fumonisins, prior to fermentation, the geometric mean of total fumonisin in maize samples were (516.96, 220.64 and 284.59μg/kg) for Ibadan, Lagos and Abeokuta respectively. After processing, the mean levels of total fumonisin in the fermented product (ogi) were (165.46, 211.77 and 150 μg/kg) for Abeokuta, Lagos and 2 Ibadan respectively. The fumonisin levels in ogi were significantly lower than the levels in the raw material (maize grain). The Wilcoxon signed rank test comparing the median levels of fumonisins before and after fermentation indicate that the difference was statistically significant in Ibadan and Lagos (**Table 2**).

Magnitude of mycotoxin reduction due to lactic acid fermentation

The percentage reduction of aflatoxins and fumonisins in maize due to lactic acid fermentation across the three locations is displayed in **Table 3**. We only focus on the reduction levels for the aflatoxins and fumonisins that were significant. Estimates were based on percentage differences between aflatoxin and fumonisin levels in the maize grain and final product (ogi). For AFB1, AFB2, AFG1 and AFG2 in Ibadan, the percentage reduction level was 48.78%, 33.33%, 37.93% and 0% respectively while for AFB1, AFB2, AFG1 and AFG2 in Abeokuta, the percentage reduction level was 74.15%, 83.19%, 46.97% and 0% respectively.

For FB1 and total fumonisins, high and significant levels of percentage reduction in maize grain from fermentation was observed in Ibadan and Lagos. For FB1 the percentage reduction level in Ibadan and Lagos were 76.67% and 81.13%. For total fumonisins, the percentage reduction levels were 66.52% and 58.90% for Ibadan and Lagos respectively). This confirms that fermentation of

maize influenced by lactic acid bacteria is associated with significant reductions in fumonisins in South West Nigeria[1]. 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 contrasts with the findings of Fandohan et al. (2005), who reported small (and statistically insignificant) effects of lactic acid fermentation on fumonisin levels (13%) in the Republic of Benin. 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.

Effect of storage and processing practices on reducing mycotoxin 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 prior to milling, because it releases bacteria 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 4** shows the mean reduction of aflatoxins and fumonisins level in ogi due to steeping duration while **Table 5** displays the results from the Wilcoxon rank sum (Man Whitney U) test that the reduction of aflatoxins and fumonisins level in ogi were different for those who steeped for the recommended number of days (2) and those who did not (in our data this would be steeping for 3 or 4 days). Across the three study locations, there is no statistically significant difference in the mean level of aflatoxin reduction due to the length of steeping in the three study locations. Though mean levels of total aflatoxin were not statistically significantly different between samples of the raw maize and the fermented product, it is still possible that the mycotoxin reduction levels for different groups of processors within a region might be different. For fumonisins, we find significantly lower reduction levels in FB3 among processors who steeped for more than the recommended days of steeping in Ibadan. The limited significant differences in mean reduction for these mycotoxins due to different lengths of steeping might be due to the limited variation in the number of days of steeping in our sample 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 to a level that would interfere with mycotoxin reduction Kpodo et al. (1996) and Okeke et al. (2015). Thus, caution against extending fermentation beyond 4 days is important.

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

Table 6 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 fewer than seven days before processing, while the

[1] We are only able to reject the null that median FB1 levels in Abeokuta are reduced in our ogi samples at P=0.109

second group included those who stored for seven or more days. We only find significant difference in reduction levels in Abeokuta, where mycotoxin reduction was higher for AFG1, FB2 and FB3 among processors who stored maize for fewer than seven days. The limited evidence of difference in aflatoxin levels between these two groups might be driven by the generally low storage periods of the maize (typically less than 2 weeks). For fumonisins, statistically significant and higher levels of reduction were observed for samples stored less than a week. Since length of maize storage is not typically associated with growth in fumonisins, this may indicate 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 8). 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 for FB1 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.

DISCUSSION

Significantly lower median levels of AFB1, AFG1, AFG2, were found in ogi – a commonly consumed processed maize porridge – after lactic acid fermentation in Ibadan. Similarly, in Ibadan and Lagos, FB1 and total fumonisin levels were significantly lower after fermentation. While median levels of other aflatoxins (apart from AFB1 and total aflatoxin) in Abeokuta were lower, these differences were not statistically significant. The geometric mean and median levels of total fumonisins in all three study locations was generally below maximum acceptable limits of 1000 μg/kg set by the European Union for maize grain (EUC, 2006). (Currently, Nigeria does not have food safety regulations for fumonisin.) However, this study found significantly lower levels of fumonisins in ogi samples in Ibadan and Lagos. This suggests that lactic acid fermentation is still able to significantly reduce the levels of this toxin, which is an important food safety finding not widely documented in the literature.

Our results for aflatoxin are consistent with Okeke et al. (2015) and Fandohan et al. (2005). However, 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 (unlike this study) did not find

a significant reduction in fumonisin levels. The geometric mean total aflatoxin level in the fermented product in the two study locations where the raw maize product had levels higher than LOD were still quite close to the maximum tolerable limit in Nigeria (also the European Union standard) 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 completely in foods after processing. This contrasts with Okeke et al. (2015) who found that fermentation reduced levels of aflatoxins and fumonisins to below LOD. Thus, the findings suggest that local foods such as fermented ogi are not necessarily 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.

Consistently, lower levels of mycotoxin 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 – what consumers are actually eating, and therefore the mycotoxin levels to which they are exposed.

While lactic acid fermentation is generally effective for fumonisin reduction, the study finds some evidence that processor practices impact the effectiveness of lactic acid fermentation. 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 length of storage and storage structures on the effectiveness of fumonisin reduction. Processors whose maize was stored less than 7 days had higher mycotoxin reduction. Significantly 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 among those used in the study area to prevent maize grain exposure to moisture, as they are well covered and placed on a cement floor.

CONCLUSION

Maize is an important staple food crop consumed all across Africa. In many parts of the continent, it is used for ogi, a porridge-like weaning food commonly used for infant / children's food 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 Ibadan and Lagos with values 66.52% and 58.90% respectively. This represents an interesting new finding for commercial lactic acid fermentation processes in reducing an

agriculturally important mycotoxin.

Even after processing, the median total aflatoxin levels in the ogi samples (in all locations where initial levels where higher than LOD) were close to the maximum acceptable limit shared by Nigeria and the European Union of 4 μg/kg at 1.3, 1.3 and 2.05μg/kg for Ibadan, Lagos and Ibadan respectively. 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 product without detectable levels of aflatoxins. 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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TABLES AND FIGURES

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
Reported problem with insects/rats/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

Note: * means conditional on storing

Table 2 Statistical analyses for total aflatoxin and fumonisin levels of maize and ogi across the groups

Groups	P-value AFB1 levels	P-value AFB2 levels	P-value AFG1 levels	P-value AFG2 levels	P-value Total aflatoxin levels
Ibadan (Before and After)	0.011**	-	0.011**	0.011**	0.011**
Lagos (Before and After)	-	-	-	-	-
Abeokuta (Before and After)	0.500	0.500	0.875	0.875	0.77
Groups	P-value FB1 levels	P-value FB2 levels	P-value FB3 levels		P-value Total fumonisin levels
Ibadan (Before and After)	0.002*	0.500	0.500		0.011**
Lagos (Before and After)	0.031**	-	-		0.031**
Abeokuta (Before and After)	0.109	0.125	-		0.109

Note:* values significant with respect to a P-value of 0.05. Sample means were compared using the Wilcoxon signed rank

Table 3: Percentage reduction of aflatoxin and fumonisin in fermented og idue to fermentation of maize.

Location	Initial level of AFB1 (µg/kg)	(%) reduction of AFB1	Initial level of AFB2 (µg/kg)	(%) reduction of AFB2	Initial level of AFG1 (µg/kg)	(%) reduction of AFG1	Initial level of AFG2 (µg/kg)	(%) reduction of AFG2	Initial level of total aflatoxins (µg/kg)	(%) reduction of total aflatoxins
Ibadan	5.76	48.78	0.3	33.33	2.9	37.93	0.4	-	9.36	41.24
Lagos	<LOD	-	<LOD	-	<LOD	-	<LOD	-	<LOD	-
Abeokuta	14.93	74.15	2.32	83.19	0.66	46.97	0.4	-	18.31	69.31

Location	Initial level of FB1 (µg/kg)	(%) reduction of FB1	Initial level of FB2 (µg/kg)	(%) reduction of FB2	Initial level of FB3 (µg/kg)	(%) reduction of FB3	Initial level of total fumonisins (µg/kg)	(%) reduction of total fumonisin
Ibadan	450	76.67	77.5	25.81	32.5	23.08	560	66.52
Lagos	132.5	81.13	25	-	25	-	182.5	58.9
Abeokuta	232.5	78.49	77.5	67.74	25	-	335	70.15

Note: FBs refers to fumonisins. AFBs refers to aflatoxins

Table 4. Mean reduction of aflatoxins and fumonisins level in *ogi* due to steeping duration.

Location	Duration of steeping (days)	Level of aflatoxin reduction (μg/kg)			
		AFB_1	AFB_2	AFG_1	AFG_2
Ibadan	2	6.20±3.39	<LOD	3.15±1.91	<LOD
	3	4.88±2.84	<LOD	2.73±1.81	<LOD
	4	6.10±0.00	<LOD	3.30±0.00	<LOD
Lagos	2	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD
Abeokuta	2	5.00±1.93	<LOD	1.30±2.25*	<LOD
	3	17.99±44.96	3.01±6.82	0.13±0.87	<LOD

Location	Duration of steeping (days)	Level of fumonisin reduction (μg/kg)		
		FB1	FB2	FB3
Ibadan	2	537.50±512.65	50.00±70.710	37.50±53.03
	3	345.83±118.76*	41.67±71.880	<LOD
	4	<LOD	175.00±0.00	<LOD
Lagos	2	89.29±116.24	<LOD	10.71±28.35
	3	150.00±198.43	<LOD	<LOD
Abeokuta	2	33.33±226.84	<LOD	<LOD
	3	246.43±315.38	75.00±109.92	<LOD

Note: * indicates means are statistically significantly different at 5%. For steeping length in Lagos and Abeokuta where there are only have two groups (2 vs 3), the study used the t- test to test for significant difference in sample means

Table 5. Statistical analysis for mean reduction of aflatoxins and fumonisins level in *ogi* due to steeping duration.

Location	aflatoxins	P-value	Z-score
Ibadan	AFB1	0.794	-0.261
	AFB2	0.617	0.500
	AFG1	0.674	-0.421
	AFG2	-	-
Abeokuta	AFB1	0.729	-0.364
	AFB2	0.207	1.262
	AFG1	1.000	0.000
	AFG2	-	-
Location	Fumonisins	P-value	Z-score
Ibadan	FB1	0.693	-0.395
	FB2	1.000	0.000
	FB3	**0.045***	**-2.00**
Abeokuta	FB1	-	-
	FB2	0.207	1.262
	FB3	-	-

Note: The Wilcoxon rank-sum (Mann-Whitney) test was used to test for significant difference in sample medians.* indicates medians are statistically significantly different at 5% or less

Table 6. Effect of length of maize storage on aflatoxin and fumonisin 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

Note: *significant at ($p<0.05$)

Table 7. Statistical analyses for length of maize storage on aflatoxin and fumonisin reduction across the groups

Location	Aflatoxins	P-value	Z-score
Ibadan	AFB1	0.253	1.143
	AFB2	0.513	0.655
	AFG1	0.139	1.481
	AFG2	-	-
Abeokuta	AFB1	0.79	-0.26
	AFB2	0.748	-0.32
	AFG1	0.05*	-1.928
	AFG2	-	-
Location	Fumonisins	P-value	Z-score
Ibadan	FB1	1.00	0.00
	FB2	0.388	0.863
	FB3	0.512	0.655
Abeokuta	FB1	0.025*	-2.236
	FB2	0.010*	-2.50
	FB3	-	-

Note: All analyses used the Wilcoxon rank-sum (Mann-Whitney) test to test for significant difference in sample medians.* indicates reduction levels are statistically significantly different at 5% or less

Table 8. 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	<LOD	1.22±7.77	<LOD	210.00±206.6	20.00±92.53	<LOD
	Plastic container	6.50±3.11	<LOD	3.82±1.84	<LOD	481.25±305.8	87.50±72.17	18.75±37.5
	Jute sack	3.80±0.00	<LOD	1.80±0.00	<LOD	175.00±0.00	<LOD	<LOD
Lagos	None	<LOD	<LOD	<LOD	<LOD	110.00±166.4	<LOD	<LOD
	Plastic container	<LOD	<LOD	<LOD	<LOD	91.67±158.8	<LOD	<LOD
	Jute sack	<LOD	<LOD	<LOD	<LOD	125.00±70.71	<LOD	<LOD
Abeokuta	None	20.55±48.28	3.37±7.40	0.65±1.59	<LOD	45.83±157.7	<LOD	<LOD
	Plastic container	7.45±10.54	0.451±0.64	0.55±0.78	<LOD	387.50±548.0**	137.50±194.5**	<LOD
	Jute sack	2.70±0.00	<LOD	1.90±0.00	<LOD	600.00±0.00	175.00±0.00	<LOD
	Polythene bag	<LOD	<LOD	<LOD	<LOD	175.00±0.00**	75.00±0.00**	<LOD

Note: * indicates means are statistically significantly different at 5%.

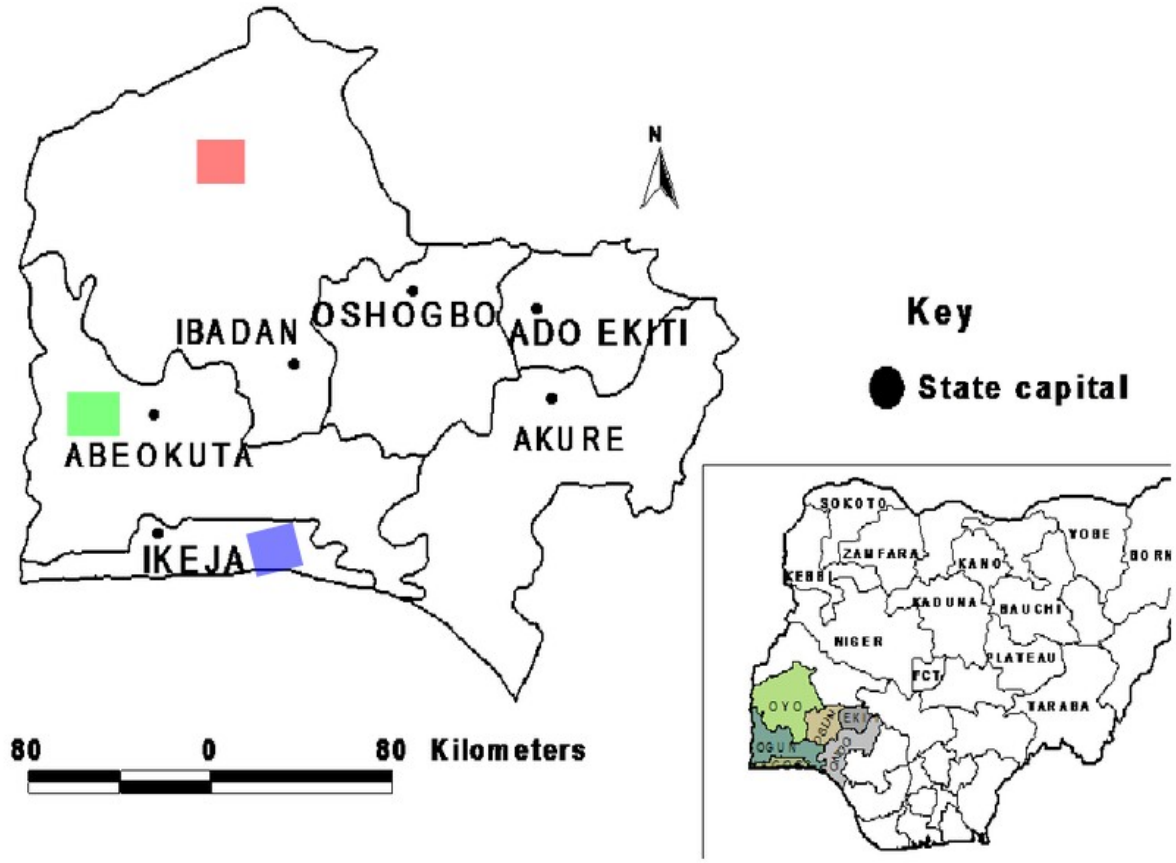

Figure 1. Map of South West Nigeria indicating the study locations.

Note: *The top left map shows the three study locations within their respective Nigerian state*[2]*. 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*** *(extracted from google inset map of Nigeria. www.researchgate.net/figure/map-of-southwest-Nigeria-showing-capital-citiesinset-map-of-Nigeria_fig1_228532647/amp)*

[2] Nigeria has 36 states (independent administrative units that constitute the Federal Republic of Nigeria) plus a Federal Capital Territory.

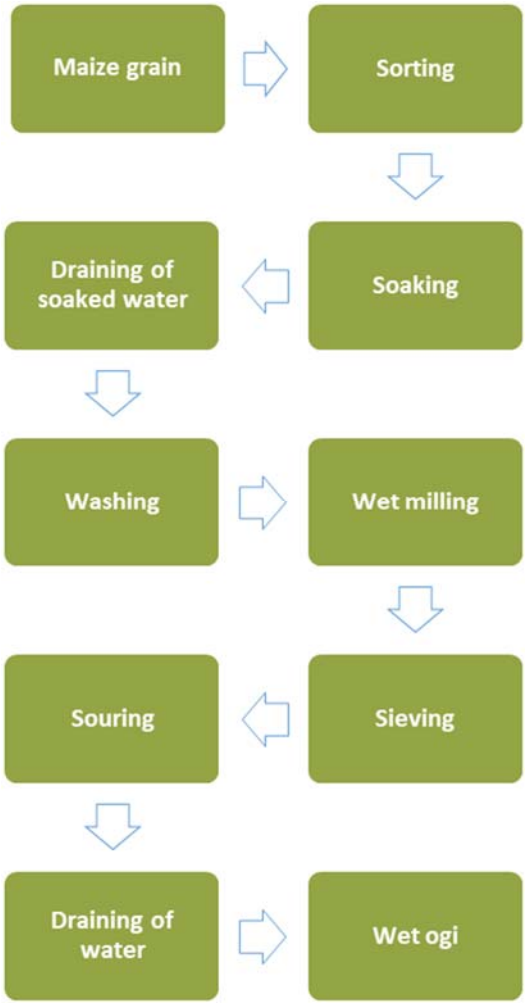

Figure 2. Flow chart of commercial processing of ogi

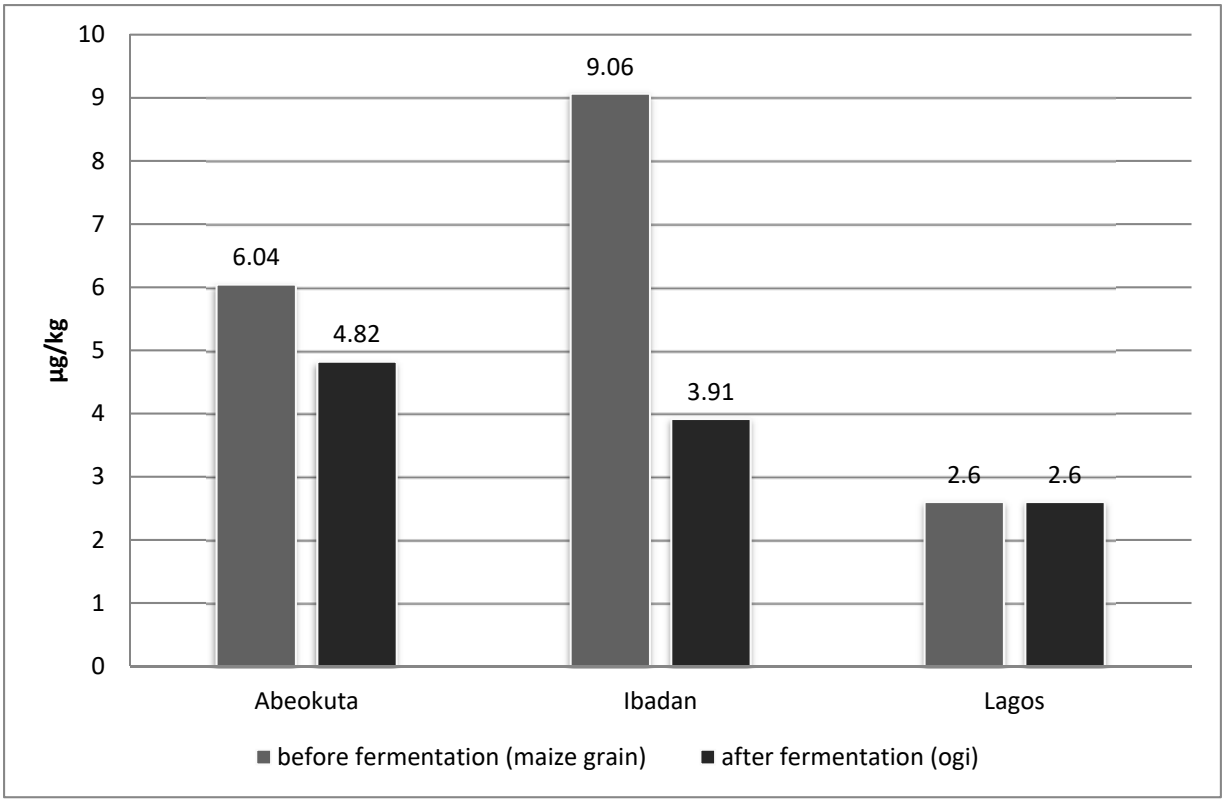

Figure 3. Geometric mean total aflatoxin levels of maize and fermented *ogi* sold in southwestern Nigeria.

Note: Total aflatoxin refers to the sum of AFB_1, AFB_2, AFG_1, and AFG_2.

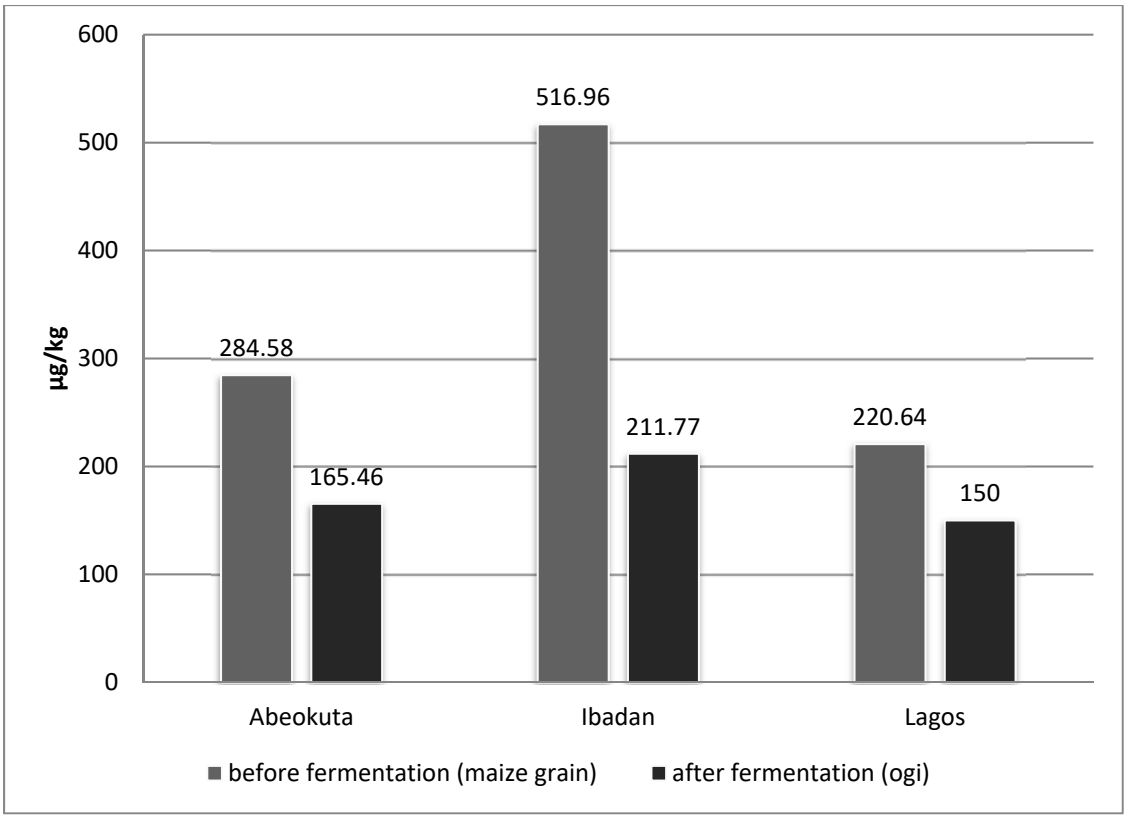

Figure 4. Geometric mean total fumonisin levels of maize and fermented *ogi* sold in southwestern Nigeria

Note: Total fumonisin refers to the sum of FB_1, FB_2 and FB_3.

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