

Aflatoxin Contents and Exposure in Young Children, and Sensory Characteristics of a Nixtamalized Supplementary Porridge

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Abstract
Aflatoxin contamination of maize, is of major concern in Malawi, and levels are much higher than the tolerance limits for aflatoxin B1 and total aflatoxins**.** Maize is a staple for Malawians and the forms prepared for consumption are presumed to have carryover toxins. *Likuni phala*; a porridge flour formulated from maize and soybean in the ratio of 4:1(w/w), was developed as a supplementary food to alleviate Protein Energy Malnutrition (PEM), which is highly prevalent in under five year old children. It is assumed that the product is contaminated with carryover aflatoxin. Nixtamalization or alkali cooking has been found to reduce aflatoxin contents among other effects. The aim of this study was to establish the levels of total aflatoxin in the *Likuni phala*, determine their reduction in porridge preparation by addition of alkali, assess the exposure to aflatoxin in small children and also test for its sensory acceptability. Samples were collected from three factories, two cottage and one large, all located in districts of the Southern Region of Malawi. Three samples were collected from each factory at two week intervals. The samples were analysed for total aflatoxin, then cooked into porridge with addition of lime at levels between 0.1% – 0.8%. The porridges were analysed for aflatoxin and subjected to sensory evaluation. Exposure to aflatoxin on the children was also calculated. Results showed that aflatoxin was absent in the samples from the cottage industries, but all the three samples from the large factory contained levels above the tolerance levels (10µg kg^{-1} for total and 5µg kg^{-1} for Aflatoxin B1). Cooking with alkali reduced the levels of aflatoxin to below the tolerance levels even at the lowest level of alkali addition. Sensory evaluation showed that the porridges were acceptable only up to 0.4% lime addition. The potential aflatoxin exposure to children was substantially reduced. The study concluded that acceptable *Likuni phala* porridge with aflatoxin levels below the tolerance levels, and substantially reduced exposure in children can be prepared by cooking with alkali addition.
Keywords: Aflatoxin, Supplemental maize porridge, Nixtamalization, acceptability, Under 5 children

1. Introduction

Malawi is one of the countries in the sub-Saharan Africa with a high dependence of maize for food security. Maize is by far the most important food staple in Malawi grown country wide and is usually processed into flour and cooked into porridge and *nsima*; a thick paste made with maize flour and water. This also implies that most complementary foods for feeding young children are made from grains and cereals. Since malnutrition has been of concern in Malawi for decades (Kalimbira *et al*., 2004), a commercial product was developed as a maize-soy blend flour from locally grown crops and named *Likuni Phala*. It is formulated in the ratio of 4 parts maize flour to 1 part soy bean flour. The product is culturally and organoleptically acceptable by many communities of the country (LaGrone *et al*., 2012). *Likuni Phala* is available in local shops, and is also distributed in specialized cases like the rehabilitation centers for PEM cases. The porridge is usually prepared according to a method recommended by the Malawi Government in its Maternal, Infant and Young Child Nutrition in Malawi Community Nutrition Workers recipe book (Malawi Government, 2011). Children 6-9 months old are usually fed 150g and children up to 3 years old are usually fed about 240g porridge per day (Malawi Government, 2011; Mphwanthe *et al*, 2016).

Aflatoxin contamination of maize in Malawi is high and studies have shown that locally stored maize contains levels beyond the acceptable threshold levels (Matumba *et al*., 2009; Matumba *et al*., 2016) of 10µg kg^{-1} for total aflatoxins and 5µg kg^{-1} for aflatoxin B1. For example, maize in stores in the Southern region of Malawi has been found to contain levels as high as 140µg kg^{-1} total aflatoxins (Mwalwayo and Thole, 2016). It can be assumed that substantial amounts of these aflatoxins is carried over to the processed product, containing maize as it's known that aflatoxins are not destroyed significantly under normal cooking processes since they have high decomposition temperatures (Betina, 1989).

Many methods have been investigated for their potential to reduce the aflatoxin contamination of maize in storage (Matumba *et al*., 2015). The toxin levels have also been used for estimation of aflatoxin exposure to consumers (Kilonzo *et al.* 2014; Kangethe *et al*. 2017). However, very few of the methods have involved, reduction of the toxin during preparation for direct consumption and also the calculation of exposure levels in the foods in their consumption state. Cooking in alkali (lime) or *nixtamalization* is a method that has been used in

South America since long ago for preparation of tortillas (Méndez-Albores *et al*., 2004). The method has been found to lower the aflatoxin levels in the nixtamalized kernels and the mash.
Alkali cooking, though not practiced in Malawi, is not new in Africa. For example, in Kenya the Kalenjin community in the Rift Valley decorticate maize with alkaline plant ashes (Imungi, 2013), the Kisiis of Western Kenya also use alkaline herbal salts to cook a maize and beans mixture called *githeri.* Many other communities in Kenya also cook maize porridge with soda ash or alkali ashes. The food cooked this way turns yellow and is also said to be tastier than that cooked without the alkali (Imungi, 2013).

This study was therefore designed to assess the effectiveness of alkali cooking in reducing the aflatoxin contamination of *Likuni phala* porridge as consumed.

2. Methodology

2.1 Sampling of Likuni phala flour

Samples of *Likuni Phala* flour were collected from three processing factories in three districts of the Southern Region of Malawi. This region has so far recorded the highest levels of aflatoxin in stored maize The factories in the districts of Zomba (DS) and Thyolo (MK) were of cottage type and were processing mainly for supply to specialized institutions like hospitals and rehabilitation centres, while the factory in Blantyre district (RL) was large and was processing for distribution to the market for access by consumers. The manufacturers were purposively selected depending whether they were in production and their willingness to participate in the study. Five kilograms of flour were sampled three times fortnightly from each processor, placed in Kraft paper bags and transported to the laboratory for aflatoxin analysis.

2.2 Likuni phala porridge preparation by Nixtamalization (alkali cooking)

Samples with higher than the 10µg kg^{-1} threshold for total aflatoxins were used. Samples of porridge were cooked with addition of 0%, 0.1%, 0.2%, 0.4%, 0.6%, and 0.8% lime on the basis of the flour and cooked according to the standard recipe adapted from Malawi Government's Infant and Young Child Nutrition (IYCN) project recipe book for Malawian Children (Malawi Government, 2011), as follows:

Four tablespoons of product were mixed with 1½ cups of distilled water in a cooking pan and brought to boil while stirring. A pinch of salt and 2 teaspoons of sugar were added and mixed thoroughly to dissolve. The heat was lowered, then the porridge simmered for five minutes and cooled to about 40^{o}C. The porridge samples were then analysed for total aflatoxins and evaluated for sensory acceptability.

2.3 Sensory Evaluation

The porridges were analysed for colour, appearance, odour, taste, mouthfeel and overall acceptance by a laboratory panel of 16 females, who were familiar with the product. A 7-point hedonic rating scale with 7 = like very much and 1 = dislike very much was used to score the product. The porridges were presented to the panellists in coded cups.

2.4 Aflatoxins Analysis

Total aflatoxin of the product and the porridges were analyzed using a VICAM fluorometer S/N: EX03150 as per manufacturer's manual following the outlined procedure for detecting aflatoxins in corn. Samples were subjected to duplicate testing.

2.5 Potential Aflatoxin Exposure Calculation

The potential exposure of the aflatoxins to the children in ng kg^{-1} body weight was calculated using the formula by WHO (WHO, 2008) as follows:

$$\text{Dietary exposure (ng/kg bw/day)}=\frac{\text{Food consumption (kg/day)}\times\text{Aflatoxin concentration (µg/kg)}}{\text{Body weight (kg)}}\times 1000$$

2.6 Statistical analysis of the data

All data was analysed using GenStat® software Version 15. Means for total aflatoxin and sensory evaluation were compared using a one-way Analysis of variance (ANOVA) and Least Significant Difference (LSD) was used to separate the means. Means for aflatoxin contents of the porridges were compared using a 2-way ANOVA and LSD was used to separate the means. All means were considered significantly different at $p<0.05$.

3. Results and discussion

3.1 Aflatoxin contents of Likuni phala

Results of total aflatoxin contents of samples collected from the three manufacturers are presented in Table 1.

Table 1. Mean total aflatoxin contents of *Likuni phala* flour by factory (on as is basis)

Sample	Total aflatoxin (µg kg^{-1})
DS1	0.0±0.0[d]
DS2	0.4±0.1[d]
DS3	0.3±0.4[d]
Mean for DS	0.4±0.3
RL1	11.9±0.7[c]
RL2	16.5±0.0[b]
RL3	33.0±5.0[a]
Mean for RL	20.5±2.0
MK1	0.0±0.0[d]
MK2	1.8±0.0[d]
MK3	0.2±0.1[d]
Mean for MK	0.7±0.0

Mean ± SD (n=3). Means along the column with the same letter superscript are not significantly different at $p<0.05$.

The aflatoxin contents of all the three samples from the large manufacturer RL were above the tolerable level for total aflatoxin of 10µg kg^{-1}. The levels of aflatoxin in samples from the two cottage DS and MK manufacturers on the other hand were all lower than the tolerance levels. The large manufacturer processes for the open market while the cottage industries process for supply to specific institutions like hospitals and nutritional rehabilitation centres. The two categories of manufacturers indicated that they usually work with the Malawi Bureau of Standards (MBS) for quality control. Furthermore, the three districts in which the factories lie are in the same agro-ecological zone of the country and therefore the maize harvest season was the same. The reason for the differences between the aflatoxin contents of the products from the cottage and the large factories is probably due to the less stringent quality control by the latter than the former manufacturers. The products from the large manufacturer were used to carry out nixtamalization studies.

3.2 Sensory Evaluation of the porridges

The mean scores for each attribute tested are shown in Table 2. The table shows that the porridges could be prepared with addition of lime up to 0.4% without significant changes ($p<0.05$) in acceptability. This level of lime would be the one recommended for addition in all products particularly at the factory level to take the nutritional advantage of calcium, an essential nutrient for growing children, for bone and teeth development.

Table 2. Sensory evaluation of nixtamalized porridge at different lime concentrations

% Lime	Attributes					
	Appearance	**Colour**	**Odour**	**Mouthfeel**	**Taste**	**Overall acceptance**
0	4.75±2.18[bc]	5.44±1.59[a]	5.94±0.77[a]	4.38±1.20[b]	5.13±1.54[a]	5.44±1.15[a]
0.1	6.13±0.96[a]	5.75±1.07[a]	5.75±1.18[a]	5.06±1.44[ab]	4.88±1.63[a]	5.06±1.39[ab]
0.2	5.81±0.83[ab]	5.81±1.28[a]	5.94±0.93[a]	5.63±0.96[a]	5.31±1.25[a]	5.5±1.21[a]
0.4	4.94±1.65[bc]	5.25±1.24[a]	4.5±2.033[b]	5.56±1.44[a]	5.38±1.63[a]	5.38±1.36[a]
0.6	4.31±1.96[c]	4.00±1.97[b]	3.88±2.31[b]	4.38±2.00[b]	4.25±2.08[a]	4.00±2.07[b]
0.8	4.25±2.21[c]	4.06±2.24[b]	3.88±2.21[b]	4.44±1.90[b]	4.19±2.11[a]	4.06±2.18[b]

Mean ± SD (n = 16) Values with same letters superscript within the column are not significantly different at $p<0.05$.

3.3 Changes in aflatoxin contents of the porridges during nixtamalization

Table 3 shows the total aflatoxin contents of the porridges cooked with lime addition. On cooking without lime, the aflatoxin content of one of the samples RL3 was still above the tolerance. The other two samples RL1 and RL2 reduce to slightly below the tolerance, probably due to dilution and slight oxidative degradation during cooking in open pan. Upon addition of lime, the aflatoxin contents dropped drastically even with addition of the lowest level of lime, and continued to drop steadily up to 0.8% lime addition. The slightly erratic rate of decrease in aflatoxin contents of the porridge with addition of lime was probably due to the errors in scooping for weighing the product for porridge preparation and sampling of the porridge for analysis, both products of which were not absolutely homogenous.

Table 3. Changes of aflatoxin contents during nixtamalization of the porridge by sample

% Lime	**Total aflatoxin (µg kg^{-1})**		
	RL1	**RL2**	**RL3**
0	6.9±0.6^{c}	9.8±0.0^{b}	19.2.±5.0^{a}
0.1	4.2±0.1^{defg} (39.1)	5.1±0.3^{de} (48.0)	4.6±0.1^{def} (75.9)
0.2	3.4±0.0^{defgh} (50.7)	4.0.±0.1^{defg} (59.2)	6.0±0.1^{d} (68.8)
0.4	1.8±0.1^{gh} (73.9)	3.2±0.1^{egh} (67.3)	1.4±0.3^{h} (92.7)
0.6	2.3±0.1^{fgh} (66.7)	4.2±0.1^{defg} (57.1)	2.9±0.0^{egh} (84.9)
0.8	2.0±0.1^{gh} (71.0)	2.1±0.0^{fgh} (78.6)	2.2±0.1^{fgh} (88.5)

Mean ±SD (n = 3). Values with same letter superscript along the columns are not significantly different at $p<0.05$, (values in parenthesis represent percent reduction in aflatoxin from the initial).

These results are comparable to those of Méndez-Albores *et al*. (2004), who reported a reduction of aflatoxin during nixtamalization of corn flour for tortillas in Mexico. Addition of 3% lime during the cooking resulted in reduction of aflatoxin B1 by 94% from 495µg kg^{-1} to 28.3µg kg^{-1}. From the two results, it appears like the destruction of aflatoxin is not dependent on the initial concentration as long as the added alkali is adequate for the amount of product. The average aflatoxin content of the porridge prepared with 0.4% lime addition fell even below the tolerance for aflatoxin B1, the most lethal of the toxins.

3.4 Potential exposure of children to aflatoxins

These calculations were based on the porridges cooked without lime addition and their respective variants that were cooked with addition of 0.4% lime; the highest level of lime addition acceptable after sensory evaluation. The calculations were done for two age groups of children; 6 – 9 months old and 30 months old; whose weights and daily porridge consumption rates were known from national figures: Body weight of rural children age 6 - 9 months, mean of 7.1kg and those at 30 months mean weight of 10.8kg (Maleta, *et al.* 2003) and daily porridge consumption rate of 150g by the former children (Malawi Government, 2011) and 240g by the latter children (Mphwanthe *et al*., 2016) . These results are shown in Table 4. The aflatoxin content of the porridge cooked without lime was 19.1µg kg^{-1}. The potential exposure to the children of age 6 - 9 months was lowered from this porridge by 82%, while for 30 month old children the potential exposure was lowered by the same percentage to the exposure of the porridge cooked with addition of 0.4% lime. Mean potential exposure to the children from the two porridges was always only slightly higher for the children 30 months than for those between 6 - 9 months.

Table 4. Potential aflatoxin exposure to the children by age

Age of Child	**Aflatoxin exposure by children's' age and sample (ng/kg bw/day)**			**Mean exposure (ng/kg bw/day)**
	RL1	RL2	RL3	
No added lime				
6-9 months	145.8	207.0	405.6	252.6
30 months	153.3	217.8	426.6	265.9
Lime at 0.4%				
6-9 months	**38.0**	**67.6**	**29.6**	**45.1**
30 months	40.0	71.1	31.1	47.4

In a study by Dhanasekaran (2011), on aflatoxins and aflatoxicosis in humans, acute aflatoxicosis was characterized by an average daily intake per adult person of 2-6 mg of aflatoxins. Acute aflatoxicosis causes serious hepatic-related sickness, leading sometimes to death (Owaga *et al*, 2011). This calculated on the basis of a 70kg adult would reduce to exposure of 37,142ng/kg body weight. For the type of children in the study, this would translate to about 4,245 ng/kg body weight, almost 100-times less than the mean exposure from the porridge cooked with addition of 0.4% lime. At this level of exposure, only chronic toxicity, (due to prolonged exposure at the sub-lethal dose), would be of concern. Chronic toxicity is associated with cancer mainly of the liver (Owaga *et al.,* 2011). Additionally in children, continued exposure to aflatoxin from diets has been reported to cause liver dysfunction, leading to apparent kwashiorkor due to inability to digest protein (Tchana *et al*., 2010), and cause impaired growth, mostly stunting (Gong *et al*., 2002, Egal *et al*., 2005). Further advanced studies involving molecular biology and in vitro animal studies are, however, required to elucidate the extent to which these exposures can cause liver dysfunction.

4 Conclusions

This study concluded that most of the *Likuni phala* that is processed for the market has levels of aflatoxin above the tolerance for aflatoxin B1 and total aflatoxins. Cooking of the porridge by the recommended method does not reduce the aflatoxins to below the tolerance levels.

Cooking the porridge with addition of lime up to 0.4% resulted in porridges which are acceptable and containing aflatoxin levels much below the tolerance levels for total aflatoxins and Aflatoxin B1.

Cooking of *Likuni phala* with addition of alkali has the potential to reduce aflatoxin exposure from consumption of the porridge by the children.

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