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Aflatoxin and Fumonisin Contamination of Sun-Dried Sweet Potato (*Ipomoea batatas* L.) Chips in Kahama District, Tanzania

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Abstract Sweet potato (*Ipomoea batatas* L.) is one of the major root and tuber crops of the tropics which can be achieved under a wide range of agro-climates and farming systems. The study was undertaken to determine aflatoxins and fumonisins occurrence in sun-dried sweet potato chips sampled from households in Kahama district in Shinyanga region, Tanzania. A total of 80 sun - dried sweet potato samples were evaluated for aflatoxins (AFB1, AFB2, AFG1 and AFG2) and fumonisins (FB1 and FB2) contaminations. High-performance liquid chromatography (HPLC) with fluorescence detection method was used for analysis. The overall percentage of samples which tested positive for aflatoxins was 36% with contamination in ranges from 10.49 µg/kg) to 75.12 µg/kg. The aflatoxin B_1 (AFBI) contamination was the most prevalent with concentration of 21.23 µg/kg followed by aflatoxin G_1 (AFGI) with concentration of 10.38 µg/kg. Fumonisin B_1 (FB1) was the only type of fumonisin detected and had 97.5% of samples contaminated with values ranging from 29.34 – 628.78 mg/kg (mean 44.69 mg/kg). Fumonisin B_1 was found to be significantly ($p < 0.01$) correlated with moisture content. The study has revealed that samples had aflatoxins and fumonisins contamination levels above acceptable levels by East Africa standards and Codex Alimentarius Commission, a joint FAO/WHO Food Standards Program. Efforts in improving post-harvest methods by addressing handling and processing methods is of prime importance in order to minimize risk of aflatoxins and fumonisins contamination of sweet potato chips.

Keywords: *aflatoxins, fumonisins, Ipomoea batatas, mycotoxin, sweet potato chips, food security*

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1. Introduction

Sweet potato *Ipomoea batatas* (L.) Lam., is a perennial dicotyledonous herbaceous plant, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. Sweet potato is one of the widely grown root crops in Sub-Saharan Africa, and expanding faster than any other food crop in Sub-Saharan Africa [1,2]. In Tanzania Sweet potato is an important subsistence food crop grown in nearly all agro ecological zones being ranked as the second to cassava in terms of root crop production; in East Africa, Tanzania ranks the second after Uganda [3,4]. Sweet potato is regarded as a food security crop mainly for of its reliability to produce adequate yields under marginal conditions. The crop also has frequently demonstrated its value as a disaster recovery crop for its ability to grow well on degraded soils under a range of rainfall [5,6].

Sweet potatoes are highly rich in carbohydrate and therefore act as a good source of energy and its young leaves and shoots are sometimes eaten as greens containing protein and crude fibre which are important for addressing deficiency diseases and colon diseases. [7,8]. The edible tuberous root is long and tapered, with a smooth skin whose colour ranges between red, purple, brown and white [9]. Sweet potatoes also serve as an excellent source of vitamin A and a good source of vitamin C, E, B6, riboflavin, pantothenic acid and folic acid, ß-carotene, iron, potassium copper and several other minerals [10,11].

The main method for processing sweet potato for storage after harvesting is through cutting into slices the roots and drying in sun where the products can last for 5 up to 10 months [12]. This traditional sun drying method normally yield poor quality since the produce is not protected against contamination with microorganisms particularly the fungi which may lead to the formation of mycotoxin [13]. Mycotoxin is a toxic secondary metabolites produced by organisms of the fungus kingdom, generally known as molds belonging to various genera such as *Aspergillus*, *Penicillium*, *Fusarium* and *Byssochlamys* [14,15]. Mycotoxin contamination is one of the factors that are a threat to food safety and quality contributing to more than 40% of the global disease burden [16].

The greatest risk to health in tropical Africa is posed by the two main type of mycotoxin namely aflatoxins and fumonisins due to their widespread prevalence and toxicity in food crops [17,18,19]. When ingested, the

mycotoxins caused by aflatoxins and fumonisins have the potential to affect human health by seriously causing acute and chronic effects such as induction of hepatocellular carcinoma (HCC), liver cancer, induction of oxidative stress, apoptosis, and cytotoxicity, as well as alterations in cytokine expression in which the acute condition may results to sudden death [20,21,22]. Although the occurrence of aflatoxins and fumonisins in food crops such as the maize and groundnuts is well documented, sporadic natural occurrence in sweet potato chips has been reported. This study was undertaken to determine aflatoxin, fumonisin in processed sweet potato chips and to unveil the levels of aflatoxins and fumonisins exposure in order to provide the information needed for promoting food safety and food security for sweet potato.

2. Material and Methods

2.1. Study Area and Sampling Procedure

The study was conducted in Kahama District in Shinyanga Region located in Lake zone, Tanzania. The region was chosen because it is one of the highest producers of sweet potatoes in Lake zone [4]. A total of 80 samples of sun dried sweet potato chips were collected in households from four villages in Ntobo Ward located between Longitude 3°38'41"S and Latitude 32°26'43"E and altitude between 1091 m -1376 m above sea level. Sweet potato in the study is mainly processed into a local product called *"Michembe"* whereby the roots are withered, cut into chips or slices and dried in sun. Approximately 200g of processed products of sweet potato chips was obtained from each household. Then samples were packed in plastic bags well labeled and sealed to maintain their conditions and transported to Tanzania Food and Drug Authority (TFDA) for laboratory analysis. The collected samples were milled by small milling machine and finally with a heavy duty blender. The milled samples were divided into three potions; one was used to determine moisture, the second for determination of aflatoxins (AFB1, AFG1, AFB2 and AFG2) and the third for determination of fumonisins (FB1 and FB2).

2.2. Determination of Aflatoxins in Dried Sweet Potato Chips

Aflatoxins (AFB1, AFG1, AFB2 and AFG2) in dried sweet potato chips in the sweet potato chips were determined by High Performance Liquid Chromatography (HPLC) with fluorescence detection. Aflatoxin was extracted for 60 min in a mechanical orbital shaker from 12.5 g of a finely ground portion of the sweet potato chips with 50 ml of methanol: water (3:2) in 100 ml ember glass bottles. The slurry was filtered through whatman No 1 filter paper. 10 ml of filtered extract was mixed with 30ml of Phosphate buffer saline (BPS) and the pH was adjusted to 7.4 using 0.1 NaOH.

The syringe barrels for receiving the sample solution were attached to the cartridge. The mixture was applied until all extract have passed over the aflatoxin strong anion exchange (SAX) cartridge (Afla Star immune affinity column) under gravity. The columns were washed with 20ml of distilled water. Any remaining water was removed from the column through a slightly negative pressure from below. The syringe barrels were removed from the columns and vials were placed under each column for collection of the eluent. Total of 1.5ml of methanol HPLC grade was applied to the column and this was directly analyzed in HPLC.

2.3. Determination of Fumonisins in Dried Sweet Potato Chips

Determination of fumonisins (FB1 and FB2) in the sweet potato was also done by determined by High Performance Liquid Chromatography (HPLC). Fumonisins was extracted for 60 min in a mechanical orbital shaker from 15 g of a finely ground portion of the chips with 40 ml of methanol:water (3:1) in 100 ml glass bottle, the flask was rinsed with 10mL of extraction solution. The mixture was filtered through Whatman No 1 filter paper and the bottle was rinsed with 10 ml of the same mixture of methanol/water, pH was adjusted to a range of 5.8- 6.5 using 0.1N NaOH. Strong Anion Exchnge (SAX) columns were placed onto the stand and the stand was attached to vaccum system. The valves of the SAX columns were closed and with an auto pipette 5ml of methanol was added into the columns. The valves were opened to ensure flow rate of about 1mL/min after that the valves were closed immediately after methanol has passed the top the columns to avoid drying of the adsorbent, the column packing.

5mL of methanol/water solution was put into the columns and allowed to flow through the column to the collection chamber. By use of auto pipette 10 ml of filtrate were applied to the columns and released. Then 8mLof methanol/water solution was put into columns followed by 3mL of methanol. Vacuum was increased to 10psi to dry the columns. The collection bowl under the columns was replaced by eluting tubes and ensured the column tips fitted well the tubes. 10mL of methanol/acetic acid was added into each column and allowed to pass at flow rate approximately 2mL/min. Then the samples were dried under nitrogen gas at 60°C.

2.4. HPLC Set up and Conditioning

A HPLC system consisting of a pump, injector, column oven, florescence detector coupled with a Kobra cell (brominated by an electrochemical cell) for magnification of AFB2 and AFG2 and its controller units was used. Chromatographic separations performed using ODS C8 column (200 X 4.6 mm, 5 µm). Mobile phase containing Methanol/Acetonitrile/water (50:20:30) mixture containing 119mg of potassium bromide and 100ul of nitric acid as the derivertising agents was used. The flow rate of the mobile phase was set 0.9ml/min, pressure 168 bar, min 2 and max 200. Fluorescence detector was set at wavelengths of 335nm excitation and 440nm cut-off emission and column oven was set at min temp 20°C and max temp 60°C. Standards were run first followed by samples. Each calibration standard was injected in triplicate; the area of each standard was recorded against its concentration.

For fumosins the HPLC system consisting of a pump, injector, column oven, florescence detector and its controller units was conditioned for 20 mins. Parameters were adjusted as follows; flow rate 1.1ml/min, temperature oven 200C, Fluorescence detector was set at wavelength

335nm excitation and 400nm emission. 200µL of standard from each point of calibration was mixed with 200µL of derivertising agent and injected within 8min. Each calibration standard was injected in triplicate; the area of each standard was recorded against its concentration. Source of standards for both aflatoxins and fumonisins were from Romer Labs®, the largest portfolio of reference materials for mycotoxins.

2.5. Moisture Content Determination

Moisture content of sun- dried sweet potato chips samples were determined using the air oven method. The weight of empty moisture dishes were recorded, then approximately 5 grams of the potato chips samples were weighed and dried to a constant weight at 105°C for 3 hours in an oven. Finally after drying and cooling desiccators for 30 minutes the weights dishes with samples were recorded. All measurements were in duplicates, the mean moisture content of the samples were calculated and expressed as percentage on weight loss.

2.6. Statistical Analysis

The analysis of variance (ANOVA) was done or the variable determined and the means separated by Duncan Multiple Range test. The correlation between the types of aflatoxins, fumonisins and the moisture content was performed using Pearson's correlation coefficient to assess the correlation between variables.

3. Results

3.1. Prevalence of Aflatoxins Contamination in the Study Area

Chromatograms of the standard and one of samples analysed for aflatoxins is shown in Figure 1 and Figure 2 respectively for intensity against retention time. The overall aflatoxins contamination of sun dried sweet potato chips ranged from was 25% and 45% across the villages in which samples were collected (Table 1). Positive samples are all analysed samples with values > Limit of detection (LOD). Ntobo na Kalangwa villages had the lowest percentage of contamination while Buganzi had the highest contamination 45%. For the overall aflatoxin contamination across the villages it was revealed that out of the 80 samples, 29 (36%) were contaminated with ranges from 10.49 µg/kg) to 75.12 µg/kg (Table 1).

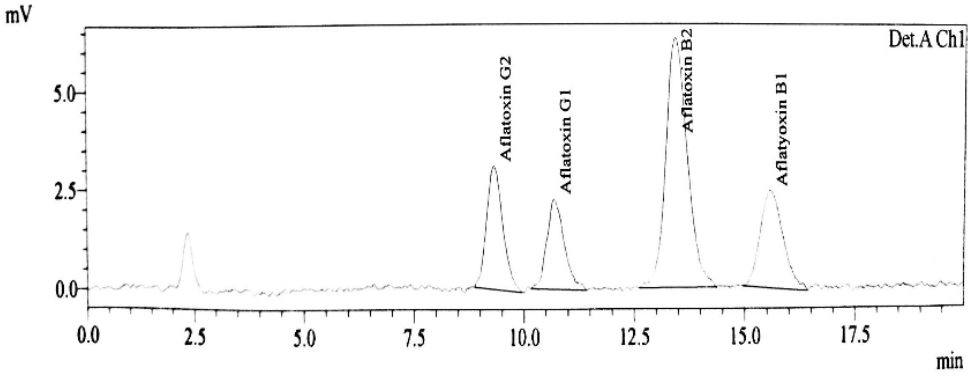

Figure 1. Chromatogram for intensity against retention time for the standard used for aflatoxins analysis

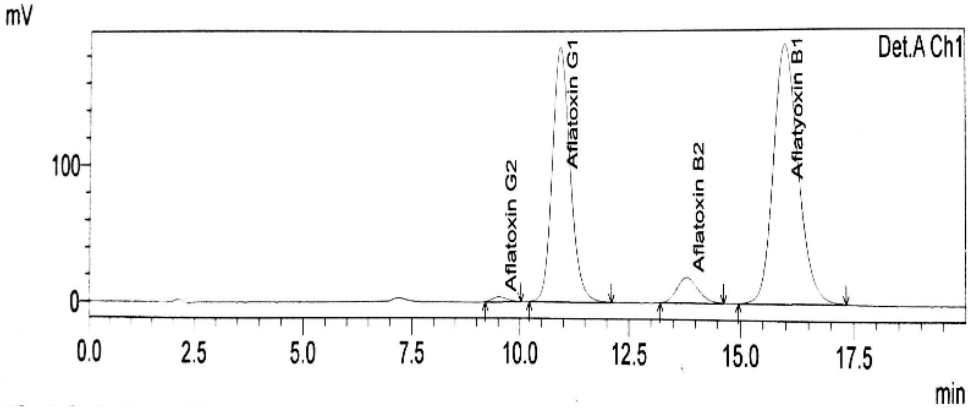

Figure 2. Chromatogram for intensity against retention time for one of the samples analysed for aflatoxins

Table 1. Prevalence of aflatoxins in dried sweet potato chips from different villages in Kahama, Shinyanga region.

Village	Samples analysed	No. of positive samples	Positive samples (%)	Range (µg/kg)
Ntobo	20	5	25	1.32 – 9.48
Kalagwa	20	5	25	0.74 – 23.97
Bukwangu	20	8	40	0.12 – 8.62
Buganzi	20	9	45	0.18 – 54.36
Total	80	29	36	10.49 - 75.12

In relation to the type of aflatoxin across the villages, a significant variation of aflatoxin types and concentration existed among the villages (Table 2). Among the villages, Buganzi village had the highest aflatoxin AFBI contamination (42.96 µg/kg) followed by Kalagwa (23.97 µg/kg). It was revealed that aflatoxin B_1 (AFBI) was the most prevalent across the sampled area compared to other types of aflatoxin.

Table 2. Prevalence of types of aflatoxins in dried sweet potato chips samples in different villages in Kahama, Shinyanga region

Village	Type of aflatoxin and concentration (µg/kg)			
	AFB1	AFB2	AFG1	AFG2
Ntobo	9.39^{a}	4.25^{ab}	8.39^{ab}	1.32^{a}
Kalagwa	23.97^{ab}	4.74^{ab}	17.31^{b}	6.24^{ab}
Bukwangu	8.61^{a}	0.72^{a}	1.05^{a}	0.12^{a}
Buganzi	42.96^{b}	9.69^{b}	14.76^{ab}	7.69^{ab}

Means in the same column with the same letter superscripts are not significantly different ($P \leq 0.05$).

Figure 3 shows the overall average of each type of aflatoxin contamination in the study area in which the aflatoxin B_1 (AFBI) was the most prevalent and had the highest concentration of 21.23 µg/kg followed by aflatoxin G_1 (AFGI) with concentration of 10.38 µg/kg . The overall percentage of aflatoxin B_1 (AFBI) contamination in all samples was 52.6%. The results (Figure 3) further show that the dried sweet potato chips were contaminated with low levels of aflatoxin B_2 (AFBI) and aflatoxin G_2 (AFG2). The total aflatoxins (AFB1+AFG1+AFB2+AFG2) varied among the villages; sweet potato samples from Buganzi had the highest total aflatoxin contamination with concentration of 77.12 µg/kg followed by samples from Kalagwa with concentration of 52.27 µg/kg where Bukwangu had the lowest concentration of 10.49 µg/kg (Figure 4). The average total aflatoxins contamination for all affected samples in the study area was 40.31 µg/kg.

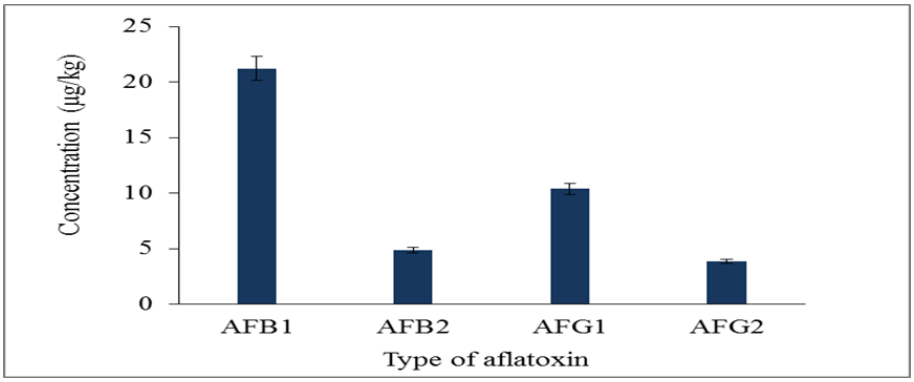

Figure 3. The overall average of concentration for types of aflatoxin contamination in dried sweet potato in the study area

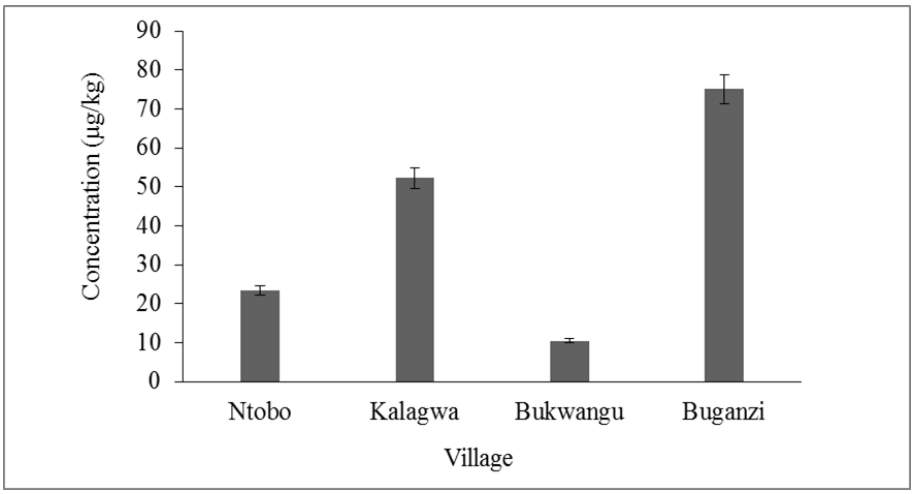

Figure 4. Variation in overall total aflatoxin contamination in dried sweet potato among the villages in the study area

3.2. Prevalence of Fumonisins Contamination in the Study Area

Chromatograms of the standard and one of samples analysed for fumonisins is shown in Figure 5 and Figure 6 respectively for intensity against retention time. The fumonisins contamination of sun dried sweet potato chips was very high ranging from 90% to 100% across the villages in which samples were collected (Table 3). Of all samples of sweet potato chips collected and analysed only fumonisin B_1 (FB1) was detected, the fumonisins B_2 (FB2) was completely absent. Generally almost all samples were found to be contaminated with fumonisins as the overall percentage of contamination was 97.5% with fumosins

contamination in ranges of concentration from 29.34 – 628.78 mg/kg (Table 3). Sweet potato samples from Kalagwa village had the lowest range of contamination with concentration 6.78 – 63.48 mg/kg while the highest concentration range 12.34 – 267.86 mg/kg was for samples collected from Bukwangu village (Table 3)

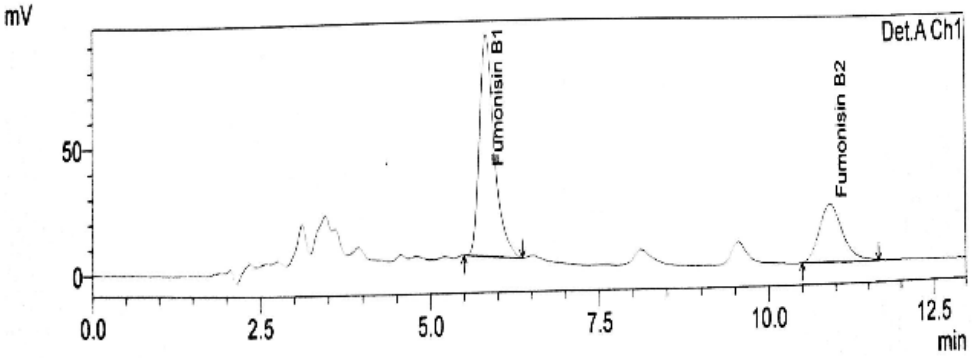

Figure 5. Chromatogram for intensity against retention time for the standard used for fumonisins analysis

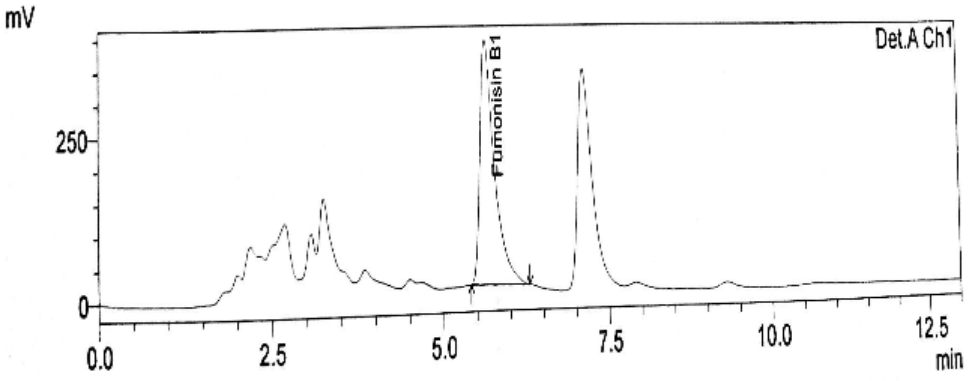

Figure 6. Chromatogram for intensity against retention time for one of the samples analysed for fumonisins

Table 3. Prevalence of fumonisin B_1 contamination in dried sweet potato chips from different villages in Kahama, shinyanga region

Village	Samples analysed	No. of positive samples	Positive samples (%)	Range (mg/kg)
Ntobo	10	10	100	15.45 – 65.82
Kalagwa	10	10	100	6.78 – 63.48
Bukwangu	10	10	100	12.34 – 267.86
Buganzi	10	9	90	8.06 – 102.74
Total	40	39	97.5	29.34 – 628.78

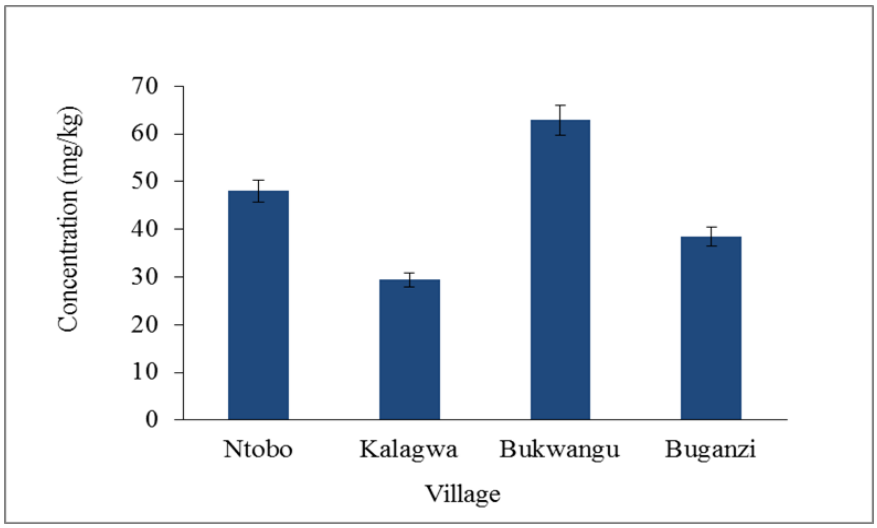

Figure 7. Variation in overall total aflatoxin contamination in dried sweet potato in villages in the study area

An overall average distribution level of fumonisin B_1 in the studied area varied across the villages (Figure 7). The Bukwangu samples showed highest average fumonisin contamination with concentration of 62.8 mg/kg followed by Ntobo samples which had fumosin contamination of 48.8 mg/kg (Figure 3). Samples from Buganzi and

Kalagwa had the low levels of detectable fumonisins when compared to other villages (Figure 7). The average total fumosins contamination for the affected samples in the study area was 44.69 mg/kg.

3.3. Moisture Content of the Samples and the Correlation

The average moisture content of the sun dried sweet potato chips slightly varied from 11.21 to 11.68% for samples collected from Kalagwa and Bukwangu respectively (Figure 8). The average moisture content for all samples in the study area was 11.42%. Correlation coefficients between aflatoxins (AFB1, AFG1, AFB2 and AFG2), fumonisin (FB1) and the moisture content revealed that significant positive correlation as well as negative correlation (Table 4). Significannt high positive correlation was found between fumonisin (FB1) contamination and moisture content s (r = 0.97). However significant negative correlations were found between all types of aflatoxins (AFB1, AFG1, AFB2 and AFG2) and moisture content (Table 4).

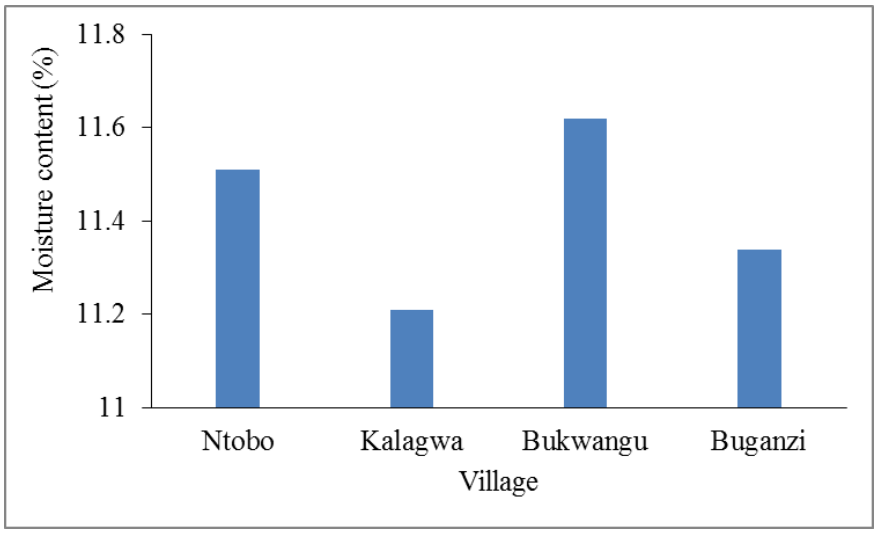

Figure 8. Variation in moisture content of dried sweet potato chips samples in the study area

Table 4. Correlation coefficients between aflatoxins (AFB1, AFG1, AFB2 and AFG2), fumonisin (FB1) and moisture content (M.C)

	AFB1	AFB2	AFG1	AFG2	FB1	M.C
AFB1	-					
AFB2	0.92*	-				
AFG1	0.73*	0.75*	-			
AFG2	0.94*	0.87*	0.91*	-		
FB1	-0.64	-0.66	-0.97*	-0.86*	-	
M.C	-0.75	-0.69	-0.96*	-0.93*	0.97*	-

*Indicate significant at $p < 0.01$.

4. Discussion

The study has revealed that sweet potato chips in Kahama district, Shinyanga region are contaminated with aflatoxins and fumonisins. For the type of aflatoxins contamination (AFB1, AFB2, AFG1 and AFG2) evaluated the study has demonstrated that aflatoxin B_1 (AFB1) contamination was the most prevalent in sun dried sweet potato chips. Aflatoxin B_1 is the most important type of the aflatoxins, considered to be the most toxic and potent carcinogen which has been directly correlated to adverse health effects, such as liver cancer. It is most unlikely that commodities will contain aflatoxins B_2, G_1 and G_2 and not aflatoxin B1. The Aflatoxins are acute and chronic toxicity produced mainly by *Aspergillus parasiticus* and *Aspergillus flavus* in tropical and subtropical regions [23,24].

For the types of fumonisins evaluated in this study, sweet potato chips were found to be contaminated only by fumonisin B_1 while fumonisin B_2 was completely absent. Fumonisin B1 is the most toxic and has been categorized as probable carcinogenic in humans and has previously been reported to have toxicological significance in the human diet [25,26]. Fumonisins are produced by a variety of fungi of the Fusarium genus *Fusarium verticillioides* or Fusarium verticillioides which commonly infect most agricultural products [22,27].

The quantity of aflatoxins and fumonisin contamination in all sweet potato chips samples were above maximum tolerable limit (MTL) for the ranges many food stuffs. The average concentration of 40.31 µg/kg total aflatoxins was above the permitted levels of 10 µg/kg by East African standards and the European Union recommended for ranges food stuffs [28,29]. Furthermore, the level of aflotoxins detected in this study is still above the accepted level by the Codex Alimentarius Commission, a joint FAO/WHO Food Standards Program which has approved a limit of 15 µg kg-1 for aflatoxins [30]. While the average total for fumonisins contamination value was 44.69 mg/kg, the value was above MTL of for ranges of

food stuffs 2 mg/kg by East Africa standards and European Union standards the limit for total fumonisins contamination [28,31].

Aflatoxin B1 has been reported to interfere with normal process of protein synthesis as well as inhibition of several metabolic systems thus causing damages to various organs especially the liver, kidney and heart [32,33]. When individuals consume foodstuffs contaminated with aflatoxin B1, the toxin is metabolized in the liver leading to formation of highly reactive chemical intermediates which when binds to DNA results in the interruption of transcription and abnormal cell proliferation, leading to mutagenesis and carcinogenesis [34]. Both aflatoxins and fumonisins exposure has been reported to suppress immune function and being among the contributing factors for impaired growth at early childhood [35,36,37].

Aflatoxins and fumonisins production may cause food spoilage and produce toxic metabolites called mycotoxins and has been related to poor food processing, environmental conditions, and lack of proper food storage facilities in developing countries [38,39]. In Tanzania particularly in Shinyanga region, sweet potato is mainly processed into a local product called "*Michembe*" whereby the roots are withered, cut into chips or slices and dried in sun on bare ground floor or on polythene bags on the ground [40]. This method plays an important role in food security as the region is prone to drought and with poor soils [40,41,42]. However, the processed food is predisposed to aflatoxins and fumonisins contamination.

The aflatoxins and fumonisins contamination depended on several parameters including moisture content, temperature, processing practices and storage facilities [27,43]. According to study done by Okungbowa and Osagie [44] on mycoflora of sun-dried sweet potato, the high moisture content of sweet potato within range of 9.0 - 9.1% after drying as well as the humidity and high temperatures encouraged the germination and colonization by fungi such as Aspergillus and Rhizopus. The moisture content of sampled sun dried sweet potato in this study was in range of 11.21 - 11.68% being above the reported levels indicating that that the products stay at moisture content that foster the growth of fungi responsible for production of mycotoxins such as aflatoxins and fumonisins. Also a significant positive correlation was found between moisture and fumonisins contamination. However, a significant negative correlation was found between moisture and aflatoxins contaminations. The finding in this study is in agreement with previously reported study for the correlation of moisture content and mycotoxins [45,46,47].

Sun drying method by the use of putting sliced potato chips on bare ground or on polyethylene sheets on the ground could be the main possible cause of contamination. The contamination could be from the micro flora on the bare ground or the polyethylene sheets. In order to reduce aflatoxins and fumonisins contamination of root tuber crops such as sweet potato, several studies have reported that sun drying method on bare ground floor has frequently resulted in high moisture content and poor product when compared to other drying surfaces such as elevated perforated surface or corrugated iron roofs/sheet [48,49,50]. In this study, though acknowledged that sun-drying method as cause of contamination, further study is needed for comparative assessment on the influence of different techniques or methods for drying sweet potato chips in relation to mycotoxins contamination. Mycotoxins contamination in sweet potato has previously been reported to be reduced by blanched solar technology for drying sweet potato chips which apart from reducing mycotoxins contamination has also been reported to have highest retention beta carotene crude fibre carbohydrate content compared to the *Michembe* methods [51].

5. Conclusion

The study established that sun dried Sweet potato chips in Kahama district in Shinyanga region is contaminated with aflatoxins and fumonisins. The study has further revealed that aflatoxin B_1 (AFB1) contamination was the most prevalent in sun dried sweet potato chips while for the case of fumonisins contamination only fumonisin B_1 was found to be prevalent in the study area. Both aflatoxin B_1 and fumonisins $B_1$are the most significant type of the aflatoxins, considered being the most toxics and potent carcinogens which have frequently being correlated to adverse health effects such as liver cancer. Improved methods and practices for processing and drying sweet potato chips can contribute to reduction of aflatoxins and fumonisins thus reducing mycotoxins in the exposure in the study area.

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