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CONSUMPTION OF AFLATOXIN CONTAMINATED PEANUT BUTTER: A HEALTH THREAT TO THE POPULATION IN LUSAKA URBAN-ZAMBIA

***Maureen S. BANDA[1], Rosemary N. LIKWA[2], Phoebe BWEMBYA[2], Jeremiah BANDA[3] and Allan MBEWE[4]**

[1]*Maureen S. Banda, C/O Amos Banda, Department of Mathematics and Statistics. University of Zambia, Box 32379, Lusaka, Zambia,* maureens.banda@gmail.com

[2]*Rosemary N. Likwa School of Public Health, Department of Community and Family Medicine. University of Zambia, Box 50110, Lusaka, Zambia likwandonyodr@gmail.com*

[2]*Phoebe Bwembya School of Public Health, Department of Community and Family Medicine. University of Zambia, Box 50110, Lusaka, Zambia pabwembya@gmail.com*

[3]*Jeremiah Banda School of Public Health, Department of Epidemiology and Biostatistics. University of Zambia, Box 50110, Lusaka, Zambia jbanda6053@gmail.com*

[4]*Allan Mbewe School of Public Health, Department of Environmental Health. University of Zambia, Box 50110, Lusaka, Zambia rabmbewe@gmail.com*

**Corresponding author e-mail:maureens.banda@gmail.com*

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Abstract: *Aflatoxin contamination is a major global Public Health problem especially in developing countries. Consumption of contaminated peanut butter poses a serious public health challenge. Aflatoxin is implicated in cancer, low immunity, and stunting among children and increases morbidity and mortality. To investigate aflatoxin contamination levels in peanut butter from local and international suppliers in selected Lusaka urban district outlets, a cross sectional quantitative study was carried out. A total of 109 peanut butter samples collected from local and international sources were tested for aflatoxin contamination levels using AccuScan Reveal Q+ test. The findings showed that nine (8.3%) of the 109 samples satisfied the 0 to 4ppb European standards, and were deemed safe for public consumption, while the majority (91.7%) were not. However, using the Codex Alimentarius Commission (CAC) standard, of up to 15ppb, 83 (76.1%) samples were found to be safe for consumption. There was a marked difference in aflatoxin contamination of products with P-value of less than 0.00001. The study revealed high aflatoxin contamination in local compared to international products at 15ppb standard where 25 local products compared to 1 product of the international origin were contaminated above 15ppb. To protect the safety of consumers, it is recommended that the regulatory agency, the Zambia Bureau of Standards (ZABS) provides guidance on standards and monitor compliance. To control the movement of contaminated peanut products across borders and their consumption, collaborative research and consumer awareness will be critical.*

Keywords*: Contamination, International, Local, products*

1. Introduction

Aflatoxin contamination is a major global Public Health threat accounting for 28% of all global liver cancer cases particularly in developing countries [1]. Several epidemiological studies in Africa and Asia have demonstrated an association between dietary aflatoxin and liver cancer. According to the International Agency for Research on Cancer (IARC) in 1988, aflatoxin B1 was on the list of human carcinogens [2]. Furthermore, the occurrence and extent of aflatoxin contamination varies and has been influenced by many environmental factors which include geographical location, suboptimal agronomic practices, susceptibility to pre-harvest fungal invasions,

transportation, storage type and processing [3,4].

Historically, aflatoxins were first discovered as a result of the deaths of over 100, 000 young Turkeys in England in 1960 from a new disease termed "Turkey X disease" [5]. This disease outbreak also occurred in more than 150 villages in Western India in 1974 where 397 persons were affected and 108 individuals died [6]. Investigations on the poultry and ducklings outbreak of "Turkey X disease" revealed that there was an association between the feed given to birds, a Brazilian peanut meal and the high toxins [7]. Since then, aflatoxin has been of interest for study among scientists throughout the world [8]. In rural Kenya, aflatoxin outbreak happened in April 2004, where 317 cases and 125 deaths of mostly children were reported because of eating aflatoxin contaminated maize [9].

When highly contaminated products with aflatoxin are consumed or just chronically eaten in low doses, health problems such as, liver cancers, aflatoxicosis, liver cirrhosis, stunted growth, low immunity and eventually death may occur [10]. Furthermore, literature reveals that highly contaminated peanut products contribute to economic loss for traders [11].

If peanut is not properly sorted by separating quality peanut from immature and discolored type before storage, there is an increased chance of moisture creation and insect manifestation [12] occurrence that increases aflatoxin levels in peanut and in turn reduces its quality. When such low quality peanut is used for peanut butter processing, aflatoxin contamination levels are likely to be raised. The Maximum Tolerable Limit of aflatoxin contamination for developing countries according to the European Union (EU) is between 4ppb and 30ppb [13]. Furthermore, legislation in the EU sets maximum aflatoxin levels for groundnuts destined for processing at 10ppb and those for direct human consumption at 4ppb [13].

In some developing countries including Zambia where limits are not yet in place, 15ppb has been prescribed as the maximum acceptable level of aflatoxin for safe consumable peanut products [14]. The presence of aflatoxin contaminated peanut butter in Lusaka's outlets may affect the nutrition status of consumers, loss of income for traders, ill health and eventually deaths of consumers. In Zambia, there is limited documentation of aflatoxin contamination that compares local with international peanut butter aflatoxin levels. Furthermore, aflatoxin contamination is not well known by many people in Zambia. However, very high aflatoxin contamination of upto 10,740ppb, way above the recommended 15ppb, have been recorded [15]. Similarly, this result may be true for both local and international peanut butter products that are sold in the outlets, a situation that predisposes consumers to dangers of aflatoxin contamination [11]. This concern led to the need for the study to investigate aflatoxin contamination in peanut butter from local and international suppliers. This is a unique study to be conducted in Lusaka, Zambia which included samples drawn from processing plants.

Peanut butter is used widely in Zambia. Results of the study may influence policy makers to enact a regulation or develop clear guidelines on how aflatoxin contamination in peanut butter can be reduced or controlled. To curb the consumption of contaminated peanut butter, the need to explore suitable interventions cannot be overmphasised. By ensuring Government and Zambia Bureau of Standards strengthen regulations to adhere to the set standards, and creating public awareness will support and safeguard the health of consumers. The objective of this paper was to investigate aflatoxin contamination of peanut butter from local and international origin.

2. Material and Methods

This study was conducted in Lusaka urban district, with a total population of 1, 747 152 [16]. There were three strata that provided cans of peanut butter for testing, one market (A), with 14 stand points, three processing plants (B, C and D), where local samples were purchased. The three commercial stores (E, F and G) represented the points from where international samples were purchased among the 20 distribution points. Cans were proportionally allocated to the stores based on the number of outlets each store had. Soweto market was chosen because of its popularity and being the cheaper supplying source for peanut butter products to customers. Similarly, Soweto was also known to have been serving as a retail market for Lusaka and beyond. The processing plants were included in order to assess aflatoxin contamination at plant level while commercial stores were included as a source for international peanut butter supply to consumers in Lusaka urban city.

Sample Selection

The suppliers (commercial store, processing plants and Soweto market) were purposively selected because they served and supplied peanut butter to the majority of consumers in Lusaka. Processing plants were identified as one of the sources for peanut butter sampling sites with the help of staff from ZABS. The managers or supervisors at the processing plants were asked to provide referrals for other processing plants where peanut butter samples could be purchased. Most of these plants were located outside of Lusaka which made them ineligible, as eligibility criteria included only those within Lusaka urban district.

Actual stores were randomly selected from the list frame. From commercial store E, one of the nine sub-outlets was selected, from commercial store F, one of the six sub-outlets and from commercial store G, one of the five sub-outlets was selected. In these selected sub-outlets, a list of peanut butter cans were developed to assist in selecting desired number. The number of cans purchased from each outlet was arrived at by the use of systematic sampling of two in one until the required number was reached. This selection was irrespective of texture or brand. However, the three processing plant owners could not allow the investigator to enter their warehouses to randomly sample units.

Peanut Butter Can Samples **Table 1**

Main Outlets	Source of Origin	Site or ID	Sample size
Local (1)	Soweto 1	A	14
	Processing Plant 1	B	12
	Processing Plant 2	C	15
	Processing Plant 3	D	14
International (2)	Store 1	E	24
	Store 2	F	16
	Store 3	G	14

Managers instead were instructed to systematically sample the number of cans that were needed which they did by picking the first and leaving the next until they had

the number required. At Soweto market, samples of peanut butter were collected from The processors were selected systematically. Purchased peanut butter samples were kept at room temperature for two weeks before taking them to the laboratory for testing. Samples were kept close to the way they were at the time of purchasing. A total of 109 peanut butter cans were purchased and of these, 55 products were local while 54 were international origin. Use of total population "N" and total sample "n" (N/n = Z) interval was used to get actual cans from sub-outlets as shown in Table 1.

Data Collection Methods

A check list was used in the outlets to collect peanut butter samples from the origin and site. Each sample was uniquely coded to avoid mix up of products drawn from different collection sites and for easy identification. A can with an identification number 2, 3 and 1 would therefore be indicating that a sample was drawn from the main outlet 2, source of origin 3, store G and sample number 1 of the 14 required. The same approach was repeated throughout the sample collection. Peanut butter was tested using Reveal Q+ Test which is a Competitive Direct Enzyme-Linked Immunosobernt Assay (CD-ELISA) in order to obtain aflatoxin concentration in parts per billion (ppb). The following materials were used: 65% ethanol solution, sample collection cups with lids, a scale, a timer, sample cup rack, Aflatoxin Stat tablet reader, graduated cylinder, filter paper, sample collection tubes with cups, 100 µL pipettes with tips, 500µL pipette, tips, paper towels and gloves. In addition, Neogen test strips, sample diluents and clear sample cups were also used.

Clear sample dilution cups were labeled and placed into a rack, 20 grams of peanut butter in cups were weighed and mixed with 60ml of 65% ethanol which was then shaken vigorously for 3 minutes in order to extract aflatoxin from the mixture. The sample was then allowed to settle for (one) 1 minute before filtration. Thereafter, 500µL of sample diluents was put in a clear cup using a 500 µL pipette to which 100µL of sample extract was added and then mixed by pipetting up and down for 5 times. Thus from this mixture, a 100 µL was later taken and transferred into a new clear sample cup where a test strip end was inserted. A timer was then set for 5 minutes to allow the strip to develop after which the strip was removed from the sample cup. Finally, the result was read using the Stat reader, which automatically analyzed the strip. The results were ready within 6-minutes of incubation.

Data Entry and Analysis

The quantitative data for aflatoxin concentration levels was entered and analysed using SPSS version 20 software package. The comparisons between local and international aflatoxin contamination was carried out using two sample t-test which was performed. In determining aflatoxin contamination, samples met specific set categories. The categories considered were 0 - 4ppb, 4 to 15 and above 15ppb. All tests were performed at 5% level of significance and 95 percent confidence intervals.

3. Results and Discussion

Aflatoxin Contamination

The results in table 2 below show the descriptive summary of findings that have been presented while infusing the discussion. The results revealed aflatoxin contamination levels to be between 1.75ppb and 147.2ppb. Generally, high level values were observed in samples drawn from local outlets while low values were from international outlets. Samples from site F had the lowest (5.3625ppb) average amount of aflatoxin while samples from site C had the highest 68.38ppb average amount of aflatoxin contamination. There were wide variations in local samples than in international samples.

Summary of Aflatoxin Contamination **Table 2**

Outlet of Origin	Sample		Aflatoxin Contamination in ppb			
	Site	No.	Mean	Min	Max	Std. Deviation
Local	A	14	32.50	3.30	147.20	46.848
	B	12	6.32	3.70	9.15	1.690
	C	15	68.38	42.90	122.50	25.104
	D	14	13.06	8.85	16.30	2.275
International	E	24	6.40	3.25	32.95	5.859
	F	16	5.36	3.95	8.10	1.194
	G	14	5.54	1.75	10.35	2.166
	Total	109	18.86	1.75	147.20	28.738

For local samples, standard deviation ranged from 1.69ppb to 46.85ppb while for the international samples, the range was from 1.19ppb at site F to 5.86ppb at site E and these were from 55 (50.5%) and 54 (49.5%) samples from local and international sources respectively. The International peanut butter products yielded lowest values of 1.75ppb to 32.95ppb while for the local products the range was between 2.275ppb and 147.2ppb.

Median Values of Aflatoxin Concentration **Table 3**

Origin	No.	Minimum	Median	Maximum	Mean	Std. Deviation
International	54	1.75	4.92	32.95	5.87	4.085
Local	55	3.30	13.10	147.20	31.62	36.066
total	109	1.75	6.95	147.2	18.86	28.738

Table 3 highlights the median for both local and international samples. The findings show that the median and the mean are reasonably close for international samples as opposed to local samples. The standard deviation of aflatoxin contamination for local sample is about nine times greater than that of the international. However, the median for samples was 6.95ppb and maximum was 32.95ppb for international products while for local product maximum was 147ppb. Of these, 25 (22.9%) samples were unsafe for human consumption. These findings were different from those cited in Malawi [17] where aflatoxin contamination for imported peanut butter had a median of 2.7µg/kg but all the local products were in the range of 34.2µg/kg to 115.6 µg/kg and these were cited as unsafe globally for human consumption. Aflatoxin contamination is a problem in many parts of the world although the values may be a little lower in Zambia where this study has revealed aflatoxin contamination levels to be in a range of 1.75ppb to 147.2ppb regardless of source of origin. Of these, 0.9% and 22.9% represented unsafe products for international and local samples respectively with over 15ppb. The findings further indicate that, regardless of the source of origin, majority (91.7%) of peanut butter samples from both local and international were contaminated as they were above the European set standard of up to 4ppb. Thus if Codex Alimentarius Commission of 0 to 15ppb is used, the results show that 83 (76.1%) of the sample as

safe for consumption. The results were found to be lower than those found in Haiti [3] where aflatoxin contamination were in the range of 137µg/kg and 2720µg/kg.

This study also shows that aflatoxin contamination is a real problem in many parts of the world, hence the need for more research to seek for interventions. This study has revealed that, peanut butter from Soweto market and processing plants in Zambia had higher aflatoxin contamination levels than products from commercial stores in contrast to the study done in Zimbabwe [18] where high aflatoxin contamination levels were detected from commercial peanut butter than from markets. In addition, another study in India [19] found aflatoxin contamination of above 30ppb with the highest values having been detected from peanut butter samples (42.5%). Moreover, the findings in this study where 26 (23.9%) samples exceeded 15mg/kg limit contrasted with the study that was conducted in Taiwan [20] where aflatoxin contamination was even higher (52.8%).

Inferential Results

A parametric approach was used to carry out the test of proportions for sample units between local and international products. Data here was found to be skewed to the right in both local and international sources (figure 1).

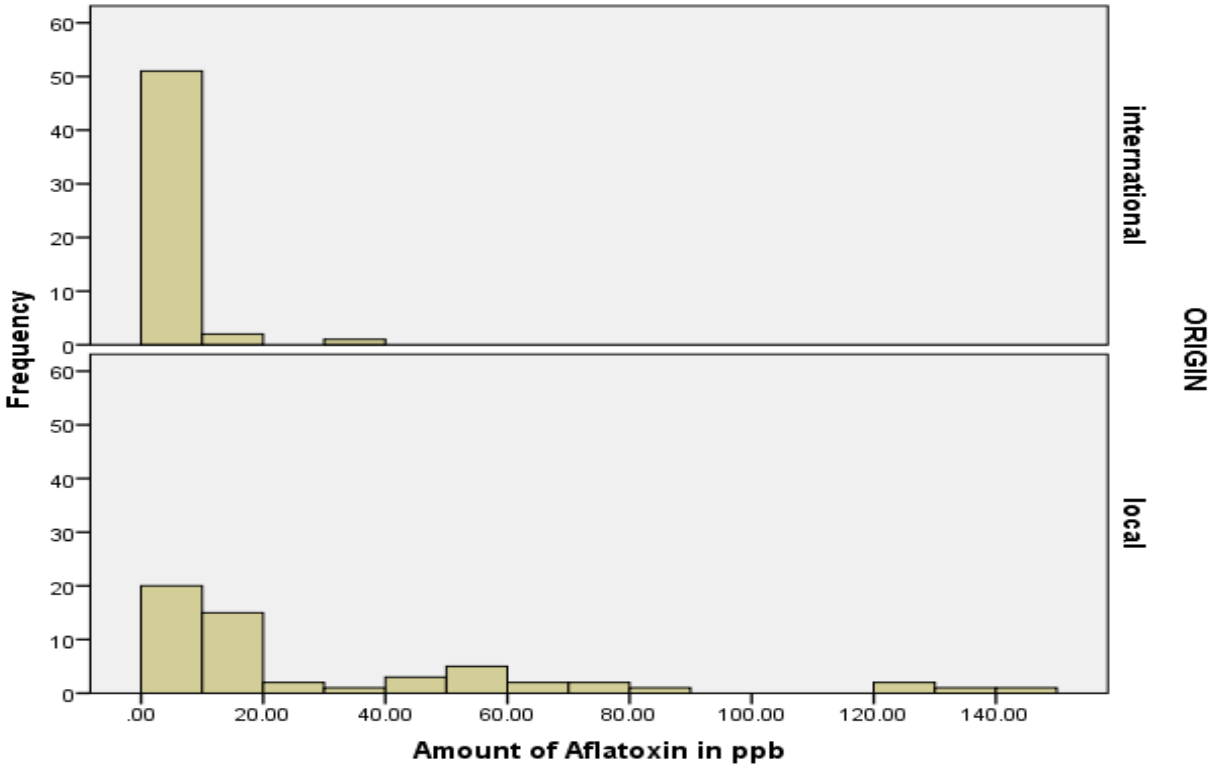

Fig 1: Aflatoxin Contamination by Source of Origin

Furthermore, variation was higher in the local as compared to the international data set. For the international peanut butter, most of the values were in the range of 0 to 10ppb while for local, they were between 0 and 20ppb with values going as higher as above 140ppb. Thus to achieve normality, the dependent variable was transformed by taking a natural log (Ln). Ln (aflatoxin in ppb) satisfied the normality assumption and a common variance could then be assumed (Figure 2).

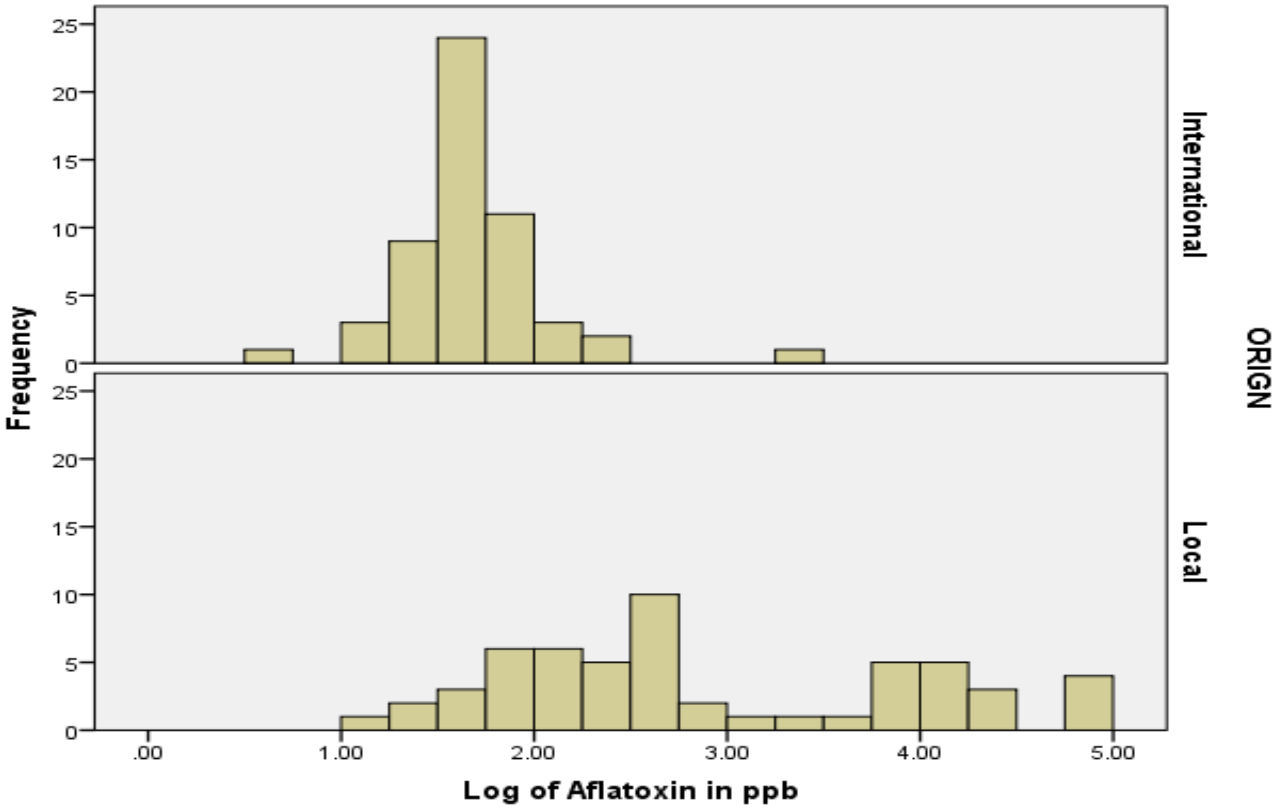

Fig 2: log of Aflatoxin Contamination

A comparison of aflatoxin contamination levels between local and international samples was carried out using the transformed variable which showed that the hypothesis that the mean values between local and international peanut butter are the same was rejected because the observed t value was -8.019 (-1.22426/0.15266) which is outside the interval (-1.98, 1.98) suggesting that the result is highly statistically significant with a P-value of less than 0.00001. Similarly, the 95% confidence interval of -1.22426 was estimated (-1.52690, -0.92163) and this also confirmed it to be significant since 0 is not contained in the interval. Further tests were carried out to compare differences in aflatoxin contamination between local and international peanut butter products.

European Standard of 0 to 4ppb

The findings in this category was that 7 out of 54 (12.96%) and 2 out of 55 (3.64%) international and local samples respectively, fell within 0 – 4ppb. Thus, the difference in proportions was -0.094 (2/55 – 7/54) with an estimated standard error of 0.053 and a Z-score value of -1.77 at 95% confidence interval of -0.011 to 0.197. This result shows that there was no significant difference (P-value = 0.0768) in aflatoxin contamination levels between local and international products according to the European set standard of 0 – 4ppb since both origins only yielded less than 10 samples as safe for consumption (Table 4).

Table 4

Proportion of Contamination in Specific Standards

Origin	Range of Aflatoxin Contamination in (%)			Total
	0 - 4ppb	> 4 - 15ppb	>15ppb	
International	7 (6.4)	46 (42.2)	1 (0.9)	54 (49.5)
Local	2 (1.8)	28 (25.7)	25 (22.9)	55 (50.5)
Total	9 (8.3)	74 (67.9)	26 (23.8)	109 (100)

Codex Alimentarius Commission 0-15ppb

This study has revealed that aflatoxin contamination at 0 to 15ppb was significantly different for local and international products at P-value of less than 0.00001. The difference in proportions was -0.43603 with an estimated standard error of 0.07081, these values yeild a Z-score of 6.158 with an estimated 95% confidence interval of 0.29566 to 0.57640. The findings in this study (76.1%) are comparable to the study conducted in Kenya [4] where 71.8% of the peanut butter products satisfied both European and Kenyan Bureau of standard as safe for consumption. The study results are contrasted with the findings reported in the survey undertaken in Zambia [15] where aflatoxin contamination in eight brands tested were in the range 73% (130µg/kg), 80% (10,740µ/kg) and 53% (1000µ/kg) respectively. Using 15ppb exposure limit to aflatoxin contamination in peanut butter, this study has revealed that local products had higher aflatoxin contamination compared to international products. This is in contrast with report that stated that South African peanut butter had the highest aflatoxin contamination than the Zambian local peanut butter products [15]. The high aflatoxin contamination in peanut butter could be attributed to the observed lack of cleanliness among traders from which peanut butter was purchased in the market. Some looked unkempt and had no protective dress code.

These findings imply that aflatoxin contamination is a problem which needs to be addressed in order to protect the population and minimise health concerns among the people. Peanut butter is one of the products that is consumed by many people in Zambia which motivate business people to buy it from Soweto market and/or other sources; and later repack it for sale after labeling the cans. The results from this study are similar to a review of Nut–Pasticcio trade [21] that stated that nations tend to trade with other nations that have identical aflatoxin standards. This led countries without legal standards to import contaminated products and supplying such to consumers. Such activities expose people to risks of aflatoxin contamination and in turn affect their health which would culminate into deaths as reported in other nations. Therefore, it is important to search for interventions that will contribute to reducing aflatoxin contamination in peanut butter for safe human consumption.

4. Conclusion

This study has revealed that aflatoxin contamination is high in locally compared to internationally processed peanut butter products based on 15ppb set standard. Using the European standard of up to 4ppb, both local and international peanut butter products

were found to be contaminated and less than ten (10) products were safe for human consumption.

This implies that the population in Zambia is consuming highly aflatoxin contaminated peanut butter products. Aflatoxin contamination seems not to be well known and as a result it is recommended that awareness campaigns be amplified to enlighten the public on the dangers of consuming aflatoxin contaminated products. Further, a recommendation to ZABS as a regulatory agency to ensure regulatory limits are set, followed and complied with by processors. Government must endorse collaborative local and international research to keep abreast on current trends of aflatoxin contamination reduction measures among stakeholders.

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