DETERMINATION OF AFLATOXIN CONTAMINATION IN CASSAVA FLOUR SOLD IN SELECTED MARKETS IN ZARIA, KADUNA STATE; NIGERIA

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ABSTRACT
Cassava (Manihot esculenta) is a major staple food crop across tropical sub-Saharan Africa. Poor drying during processing or storage, especially during the rainy season, often results in contamination by fungi such as Aspergillus, Fusarium and Penicillium. Aflatoxins are among the mycotoxins which are secondary metabolites produced by some strains of Aspergillus flavus and Aspergillus parasiticus which are a major health concern to man and livestock because of their acute and chronic health effects. The aim of this study was to determine aflatoxin contamination and to quantify the aflatoxin levels in cassava flour. A total of 36 samples of Cassava flour were collected in three (3) major markets in Zaria, these are: Samaru, Sabon Gari and Zaria City Markets to determine and quantify aflatoxins using enzyme linked immunosorbent Assay (ELISA). The results obtained showed that 22 out of 36 were contaminated with total aflatoxins at the range of 2.0µg/kg to 7.5µg/kg. This study indicated that the is low aflatoxin concentration in the Cassava and suggested that the cassava flour is safe and good for human consumption.

Key words: Cassava, Aflatoxin, Fungi, Market

INTRODUCTION
Cassava (Manihot esculenta) is a major staple food crop across tropical sub-Saharan Africa (FAO, 2012). It has huge potential for industrial application in the plywood, confectionery, feed and pharmaceutical sectors (Moyo, et al. 2004; Kaaya and Eboku, 2010). However, poor drying during processing or storage, especially during the rainy season, often results in contamination by fungi such as Aspergillus, Fusarium and Penicillium that produce mycotoxins. Aflatoxins are among the mycotoxins which are secondary metabolites produced by some strains of Aspergillus flavus and Aspergillus parasiticus, aflatoxins and are a major health concern to man and livestock because of their acute and chronic health effects. Aflatoxins poses a greatest risk to health in tropical Africa because of their widespread prevalence and high toxicity (Manjula, et al. 2008) and have been known to be carcinogenic (cause liver and esophageal cancer), immune system suppressing and anti-nutritional contaminants in many food commodities and even to cause death (Jiang, 2005).

There are four naturally occurring aflatoxins, designated B₁, B₂, G₁ and G₂ with AFB₁ being the most common toxin (IARC, 1993). Aflatoxin B₁ is produced by both Aspergillus flavus and Aspergillus parasiticus and aflatoxin G₁ and G₂ are produced exclusively by A. parasiticus. Aflatoxin M₁ and M₂ were originally discovered in the milk of cows that fed on moldy grains and also aflatoxins M₁ are metabolite of aflatoxin B₁ in humans and animals while aflatoxins M₂ are metabolite of aflatoxin B₂ in milk of cattle fed on contaminated foods (Boutrif, 1998).

MATERIALS AND METHODS
Sample Collection
A total of 36 samples of Cassava flour were randomly collected inside clean polythene bag at each point of sale in three (3) major markets in Zaria, these are: Samaru, Sabon Gari and Zaria City Markets and transported to the laboratory for the department of microbiology Ahmadu Bello University, Zaria for further analysis.

Sample Processing and Preparation
Fifty gram (50g) of each sample (cassava flour) and 5.0g NaCl were transferred to a clean blender jar and 100ml of 80% methanol/water were added to the jar and blended for 1 minute in a high speed blender. The solution was filtered through a filter paper (coffee filter) and then 5ml of extract was diluted with 20ml of water, the mixture then were filtered through a glass fiber filter and the extracts were used for aflatoxin levels.

Determination of the Aflatoxin Levels in the Samples
The aflatoxin levels in the samples were determined using enzyme linked immunosorbent Assay (ELISA). According to the Beacon manufacturer’s instruction aflatoxin test kit. Reagents and samples extracts were allowed to reach room temperature prior to starting the test.
The appropriate numbers of test wells were placed into a microwell holder and 50µl of enzyme conjugate were dispensing into each of the test well. Another 50µl of calibrators and sample extracts were added to the appropriate test wells by using clean pipette tip for each and also 50µl of antibody solution were added into each test. The plate were gently shaken to mix the contents and incubated for 10 minutes. The contents of the wells were washed into an appropriate waste container by overflow to fill the wells with laboratory quality distilled water and were repeated 4 times for a total of five washes. Following the last washing the wash solutions were removed by inverting the wells onto absorbent paper and 100µl of substrate was added into each well and shaken gently. Followed by incubation for 10 minutes, this was followed by addition of 10µl of stop solution into each well and shaking the plate rack gently to mix. The absorbance of the wells were read at 450nm using an ELISA reader and the printed results was interpreted quantitatively by graphing the absorbances of the calibrators (Y-axis, LOGI) versus the calibrator concentration (X-axis, LOG) on LOG-LOGIT graph paper. A straight line was drawn through the calibrator points and the sample absorbance was located on the line. The corresponding point on the Y-axis is the aflatoxins concentration (µg/kg) of the sample.

RESULTS
Aflatoxin concentration in flour from Samaru Market indicated 8 out of 12 Cassava were contaminated with the total aflatoxin 2.0µg/kg to 7.5µg/kg and also a total aflatoxin concentration in flour from Sabon gari Market, 3 out of 12 Cassava flour were contaminated with 2.0µg/kg respectively (Table 1). However, the total aflatoxin concentration in sample from Zaria city Market revealed 11 out of 12 Cassava flour were contaminated with 2.0µg/kg to 7.5µg/kg as shown in Table 1.

The statistical package for social sciences (IBM SPSS statistics21) was used for the analysis of variance of mean of aflatoxin concentration of all the three markets, Samaru 1.80, Sabon gari 0.50 and zaria city 3.21 as presented in table 2. Statistically between Zaria-Samaru (p=0.009) and Zaria-Sabo (p=0.002) are significant while between Samaru-Sabo markets are not significant (p= 0.123) (Table 2).

Table 1: Comparison for Aflatoxin Concentration in Cassava Flours from Three Markets

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>SAMARU AFLATOXIN (µg/kg)</th>
<th>SABON GARI AFLATOXIN (µg/kg)</th>
<th>ZARIA AFLATOXIN (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>B</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C</td>
<td>0.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>D</td>
<td>0.0</td>
<td>0.0</td>
<td>7.5</td>
</tr>
<tr>
<td>E</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>F</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>G</td>
<td>7.5</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>H</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>I</td>
<td>2.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>J</td>
<td>2.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>K</td>
<td>2.0</td>
<td>0.0</td>
<td>7.5</td>
</tr>
<tr>
<td>L</td>
<td>0.0</td>
<td>0.0</td>
<td>7.5</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>1.80</td>
<td>0.50</td>
<td>3.21</td>
</tr>
</tbody>
</table>

Table 2: Analysis of Variance of Mean of Aflatoxin Concentration from the Three Markets

<table>
<thead>
<tr>
<th>MARKETS</th>
<th>MEAN OF AFLATOXIN (µg/kg)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMARU- SABO</td>
<td>1.80-0.50</td>
<td>0.123</td>
</tr>
<tr>
<td>ZARIA-SAMARU</td>
<td>3.21-1.80</td>
<td>0.009</td>
</tr>
<tr>
<td>ZARIA- SABO</td>
<td>3.21-0.50</td>
<td>0.002</td>
</tr>
</tbody>
</table>

DISCUSSION
Aflatoxins in the agricultural produce could not be avoided completely but would be reduce to acceptable limits. There is no safe level for aflatoxin for human consumption but most of the countries including Nigeria taking into account global standard to regulate aflatoxin levels in all food at 20ppb (FAO, 1997). The regulation is because to reduce the chance of occurrence of serious health related hazard due to the ingestion of high level of aflatoxins in food such as hepatocellular carcinoma (HCC- Liver cancer) (Liu and Wu, 2010. A total of 36 samples of Cassava flour were tested for total aflatoxin concentration. This study revealed that the aflatoxin contamination in cassava flour ranged from 2.0µg/kg to 7.5µg/kg. These was below the maximum acceptable limited of 10µg/kg stated by Codex Alimentarius Commission, (1995) of the UN and suggested by NAFDAC in Nigeria (Atanda, 2005). This result is in agreement with that reported by Chiona, et al. (2014) at Malawi and Zambia the level of fungal and mycotoxins contamination in commonly processed Cassava products. It also agrees with Bankole, et al. (2006) who reported that Cassava and Yam are not vulnerable to aflatoxin contamination.
However, this result showed that 14 out of 36 samples were not contaminated with aflatoxins while those contaminated had less than 10µg/kg. This may be due the fact that Cassava flour does not provide a good substrate for aflatoxin production (Wood, 1992) and however, the analysis of variance of mean of aflatoxin concentration of all the three markets revealed that statistically between Zaria-Samaru (p=0.009) and Zaria-Sabo (p=0.000) are significant while between Samaru-Sabo markets are not significant (p = 0.123), this may be due to the fact that difference in storage techniques and ecological conditions as supported by Roy and Chourasia (1990).

CONCLUSION
This study revealed that there is low aflatoxin concentration in the Cassava flour from the study area which suggested that the cassava flour is safe and good for human consumption.

CONTRIBUTIONS OF AUTHORS
Based on the findings my contributions are:
These findings revealed that the cassava flour are safe for human consumption as at that period of the study and also this finding will be use for future literature and serve as an avenue for further research due to the fact that there is little or no available record related to presence of aflatoxins in cassava flour in the study area.

CONFLICT OF INTEREST
The conflict of interest is none

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REFERENCES


