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Evaluation of Aflatoxin Content in Tomatoes (*Lycopersicum esculentum)* in Awka

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ABSTRACT

Healthy and unhealthy tomatoes were analyzed to compare the aflatoxin content and evaluate heat treatment on the unhealthy tomato samples in Awka. The healthy and unhealthy tomato samples were selected randomly from Eke Awka Market, First Market and Second Market. The aflatoxins were extracted and analyzed by using Thin Layer Chromatography and effect of heat treatment on aflatoxin levels of the samples were determined after 10, 20, 30minutes of heat treatment at 100^{0}C. The results showed that the presence of aflatoxin in the healthy tomato samples was below the limit of detection (0.005µg/kg), while in the unhealthy tomato samples it was above the limit of detection. The average value of aflatoxin contents in unhealthy tomato samples were 8.02 $\pm$ 0.43, 7.94 $\pm$ 0.89, 8.49 $\pm$0.51 respectively. The prevalence and amount of aflatoxin was highest at second market and lowest at first market. The effect of heat treatment on the aflatoxin contaminated unhealthy tomato samples at 100^{0}C for 10, 20 and 30 minutes significantly reduced the aflatoxin levels to 35.33%, 51.83%, 71.85% respectively. Therefore, the consumption of healthy tomato is safer than the consumption of unhealthy tomatoes and proper cooking of tomatoes at 100^{0}C under normal atmospheric conditions over a period of time will significantly reduce the level of aflatoxin in tomatoes.

Keywords: Aflatoxin, *Lycopersicum esculentum*, Heat treatment

INTRODUCTION

Aflatoxins are a class of secondary metabolites of fungal origin that are toxic, mutagenic, teratogenic, and carcinogenic. They are produced by various species of Aspergillus, including *A. flavus*, *A. parasiticus*, and in rare instances, *A. nomius* and *A. pseudotamari* [1], [2]. They are particularly harmful to humans and animals because they are carcinogenic because they are linked to hepatocellular carcinoma, which eventually results in liver cancer [3]. Aflatoxin pollution affects a variety of local crops and food products. The majority of aflatoxins contaminate food and feed, including corn, rice, spices, dried fruits, nuts, and figs [4]. Aflatoxin is the main mycotoxin in food that eventually compromises the health of humans and animals among those that impact food and feed. Aflatoxin pollution affects approximately 4.5 billion people worldwide due to its pervasiveness [5]. Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) are the four primary aflatoxins, while there are more than 20 known. However, the hydroxylated metabolites of AFB1 and AFB2 are aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2). Aflatoxin toxicity varies according to the types present; AFTs-B1 > AFTs-G1 > AFTs-B2 > AFTS-G2 is the order of toxicity [6]. Although *Aspergillus flavus* and *Aspergillus parasiticus* are the most common producers of aflatoxins, Emericella astellata and E. venezuelensis from Nidulatans, as well as *A. nomius*, *A. pseudotamarii*, *A. parvisclerotigenus, and A. bombycis from section Flavi*, *A. ochraceoroseus*, and *A. rambellii* from section Ochraceorosei, have also been identified as aflatoxins [5]. Consuming aflatoxins can result in the disease known as aflatoxicosis [7]. Aflatoxin-producing fungi are found around the world and can contribute to the microflora of

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vegetables and fruits as unintentional contaminants from the soil, dust, and environment, or as a result of unhygienic handling methods and subpar farming standards such as tomatoes and other vegetables [8]. The tomato (*Lycoperscium esculentum*) belongs to the Solanaceae family of herbaceous plants. For microbes as well as humans, tomatoes are a good source of nutrition due to their rich nutritional composition. Due to their fragile skin, these fruits are extremely vulnerable to fungal contamination, primarily through damaged or injured tissues [9]. They are extremely susceptible to microbial infection due to their sensitive skin. Tomatoes are particularly vulnerable to parasite infection during field transit, preparation, and storage; insects that are wound in the plant induce stress and provide as a breeding ground for aflatoxigenic fungus [9]. Tomato spoilage refers to unfavorable alterations in tomato quality caused mostly by biological and physical processes. Because of these modifications, tomatoes are no longer fit for human eating. Areas of infection are not the only places where aflatoxins can be found. These aflatoxins spread quickly throughout tomatoes due to their high fluid content, infecting every part of the fruit and rendering it unsafe for human consumption. In Nigeria, using unhealthy tomatoes as stew ingredients is a popular habit, particularly among the lower and middle classes, without taking into account the possible health risks [10]. Among other things, socioeconomic, cultural, and belief variables may be to blame for these. Nigeria's poverty rate has been among the highest in the world in recent years [10]. People's lives could be in jeopardy if they eat unhealthy tomatoes that contain aflatoxin. The usage of spoiled tomatoes, also referred to as "awarawa tomatoes," in food preparation has been extremely concerning in Awka, one of Nigeria's emerging cities. According to recent reports, eating spoiled tomatoes may be directly related to the increased risk of cancer in humans [2]. This study in assessing the aflatoxin concentration of tomatoes (*Lycopersicum esculentum*) in Awka was prompted by the difficulties associated with eating spoilt tomatoes.

MATERIALS AND METHODS

SAMPLE COLLECTION

Tomato samples (Plate 1 and Plate 2) were procured from three market namely, Ifite First Market, Ifite Second Market and Eke-Awka Market.

EXPERIMENTAL EQUIPMENTS

The equipment's used include, weighing balance, electric blender, beaker, separation funnel, conical flask, measuring cylinder and timer clock

EXPERIMENTAL PROCEDURE [11]

SAMPLE PREPARATION

For comparative analysis: Fifteen grams (15g) of healthy and unhealthy tomatoes samples 9 each (3 each from the different sampling area) were grounded into paste and extracted using a suitable solvent (acetonitrile). The extract was then transferred into a beaker and ready for analysis.

Heat treatment: Fifteen gram(15g) each of 9 unhealthy tomatoes samples were boiled at different temperature and time period of 10min, 20mins and 30min respectively. The sample were further grounded into paste and extracted using a suitable solvent (acetonitrile). The extract was then transferred into a beaker and ready for analysis.

THIN LAYER CHROMATOGRAPHY (TLC) PLATE PREPARATION

A thin layer of a suitable adsorbent material, such as silica gel was coated on a glass plate. The plate was then activated by heating in an oven.

DEVELOPING THE TLC PLATE

The TLC plate was placed in a developing chamber containing a suitable solvent system, such as chloroform-methanol-water, which allows the different components of the sample to migrate up the plate at different rates.

SPOTTING THE SAMPLE

1ml of the extract was spotted onto the TLC plate using a micropipette.

STANDARD CALIBRATION PREPARATION (Method of *[11]*

To generate a calibration curve for the determination of aflatoxin levels using the TLC method, a series of known concentrations (0.5, 1.0, 2.0, 4.0, and 8.0 µg/mL) of aflatoxin standards were prepared and spotted onto the TLC plate along with the sample extract. The spots were then developed and visualized, and the intensity of the aflatoxin spots was measured. The concentration of aflatoxin in the sample was then determined by comparing the intensity of the aflatoxin spot in the sample with the intensity of the spots generated from the standard solutions of known concentration.

AFLATOXIN VISUALIZATION:

The Method of *[11]* was used. After the plate was developed, it was removed from the developing chamber and allowed to dry. The aflatoxin spots were then visualized using a suitable detection reagent (iodine vapors and UV light.)

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QUANTIFICATION

The amount of aflatoxin in the sample was determined by measuring the intensity of the aflatoxin spots and compared to a calibration curve generated using known concentrations of aflatoxin standards. Then the Aflatoxin B1, B2, G1, and G2 was identified and their concentrations recorded accordingly.

Plate 1: Healthy tomato samples

Plate 2: Unhealthy tomato samples procured from First market

RESULTS

AFLATOXIN CONTENT OF HEALTHY AND UNHEALTHY TOMATO SAMPLES IN AWKA

In the healthy tomato samples, the aflatoxin content was below the limit of detection (LOD) value 0.005 µg/kg for all the samples analyzed from sample areas Eke-awka (E), Ifite First market (F) and Ifite Second market (S)

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respectively (Table 1). While the average value of aflatoxin contents in the unhealthy tomato samples was E (8.02 ± 0.43), F (7.94 ± 0.89), S (8.49 ±0.51) respectively (Table 1). The amount of aflatoxin in the unhealthy tomato samples was highest at Ifite Second market (8.49 ± 0.51) and lowest at Ifite First market (7.94 ± 0.89) though statistically there were no significant difference in the values obtained from the samples ($p>0.05$).

Table 1: Average aflatoxin content of healthy and unhealthy tomato samples from Eke- Awka, First market and Second market

Sampling type	Sampling area	Average aflatoxin level (µg/kg)
Fresh	Eke-Awka	<LOD
Fresh	First market	< LOD
Fresh`	Second market	< LOD
Spoilt	Eke-Awka	8.02 ± 0.53^{a}
Spoilt	First market	7.94 ± 0.89^{a}
Spoilt	Second market	8.49 ± 0.51^{a}

Results are expressed as means + standard deviation of triplicate analysis; Means value the same superscript show no significant difference; LOD = Limit of detection = 0.005; significance level = ($p \leq 0.05$).

HEAT TREATMENT ON UNHEALTHY TOMATO SAMPLES AT 100°C FOR 10, 20 AND 30 MINUTES

After heat treatment at 100°C for 10, 20 and 30 minutes, there was a significant level of reduction of aflatoxin in values gotten from the spoilt tomato samples from Eke-Awka market, First market and Second Market (Table 2). After boiling at 100°C for 10 minutes the average values obtained were E (5.51±0.23), F (5.13±0.14), S (5.78±0.56), After 20 minutes, the average values obtained were E (4.16±0.19), F (3.91±0.21), S (4.21±0.58) and after 30 minutes, the average values obtained were E (2.68±0.36), F (1.89±0.06) and (2.61±0.2). The average difference among the values obtained after heat treatment; 5.47 ± 1.29, 4.09 ± 0.58 and 2.39 ± 0.61 (Table 3) showed a reduction level of 35.33%, 51.83% and 71.85% respectively.

Table 2: Aflatoxin content after heat treatment on unhealthy tomato samples at 100°C for 10, 20 and 30 minutes

Sample Area	Treatment period (minutes)	Aflatoxin level (µg/kg)
Eke-Awka	10	5.51±0.23
First market	10	5.13±0.14
Second market	10	5.78±0.56
Eke-Awka	20	4.16±0.19
First market	20	3.91±0.21
Second market	20	4.21±0.58
Eke-Awka	30	2.68±0.36
First market	30	1.89±0.06
Second market	30	2.61±0.2

Results are expressed as means + standard deviation of triplicate analysis; Means value the same superscript show no significant difference; LOD = Limit of detection = 0.005; significance level = ($p \leq 0.05$)

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Table 3: Average heat treatment efficiency on aflatoxin level of spoilt tomato sample

Treatment period (minutes)	Average Aflatoxin level (µg/kg)	Heat treatment efficiency (%)
10	5.47 ± 0.46^{a}	35.33
20	4.09 ± 0.23^{b}	51.83
30	2.39 ± 0.19^{c}	71.85

Results are expressed as means + standard deviation of triplicate analysis; Means value the same superscript show no significant difference; LOD = Limit of detection = 0.005; significance level = ($p \leq 0.05$)

DISCUSSION

This study shows the presence of aflatoxin in the healthy tomato samples were below the limit of detection (0.005µg/kg), while in the unhealthy tomato samples the presence of aflatoxin was above the limit of detection. Comparatively the values gotten from the results were below the maximum allowable limit (10 µg/kg) for human consumption as the values adopted by National Agency for Food and Drug Agencies in Nigeria for raw food [12]. Aflatoxin is most harmful to humans and animals as it is carcinogenic due to its association with hepatocellular carcinoma which leads to liver cancer and other human diseases [13]. The study also shows that the occurrence of aflatoxin in the unhealthy tomato samples vary according to geographical location (market). In this study the prevalence and amount of aflatoxin was highest at second market and lowest at first market, even though statistically there was no significant difference. This might be due to storage in unhygienic condition [14]. According to [14], temperature, humidity, environmental stress, injury caused by insects or birds on the host, and post-harvest practices are some of the factors that promote aflatoxin production. The environment and method of handling food samples in Ifite Awka second market in this study is poorer when compared to other markets. This may explain why the prevalence and amount of aflatoxin is higher in Ifite Awka second market. Physical methods such as steam under pressure, dry roasting, and other cooking methods are found to be effective in the control or to reduce the aflatoxin contamination in many crops [15]. About 40–73% reduction in aflatoxin level was also observed by heating the food samples on temperature from 100°C - 180°C [16]. This study showed that the effect of heat treatment on the aflatoxin contaminated unhealthy (spoilt) tomato samples at 100°C for 10, 20 and 30 minutes significantly reduced the aflatoxin levels to about 35.33%, 51.83%, 71.85% respectively. This shows that proper cooking of tomatoes at 100°C under normal atmospheric conditions over a period of time will significantly reduce the level of aflatoxin in tomatoes. This has proven to be an effective strategy in reducing aflatoxin level in food samples.

CONCLUSION AND RECCOMMENDATION

Aflatoxins contamination in food crops and their derived products have become a serious threat to humans and animal's lives. Although, the level of aflatoxin in the unhealthy tomato samples where below the maximum allowable limit (10µg/kg) for human consumption and heat treatment significantly reduced the level of aflatoxin in the unhealthy tomato samples in this study, it is safer for people to consume healthy tomatoes and avoid consumption of unhealthy tomatoes entirely. Also, good and sanitary practices should be employed especially during harvest by placing their harvested produce in clean containers. Sanitary practices should also be employed at selling points. Since tomatoes are perishable products, good storage conditions should be considered to decrease the chances of rapid contamination by fungi which is the cause of aflatoxin contamination. Lastly, regulatory agencies such as NAFDAC should ensure regular monitoring of aflatoxin contamination in foods and feeds.

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