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Determination of Aflatoxin in Fresh, Semi-Rotten and Dried Tomatoes in Dekina Local Government Area, Kogi State, Nigeria

Suleiman Mohammed Salisu Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria

Ojogbane Ele-Ojo Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria

Omede Ameh Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria

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Over the years, aflatoxin contamination of foods and vegetables have been a serious threat to the wellbeing of animals and food security in resource poor nations. The aim of the research is to determine the level of aflatoxin contamination of tomatoes (Solanum lycopersycum) marketed within Dekina local Government Area of Kogi State, Nigeria. Forty-five (45) tomatoes samples consisting of (15 fresh, 15 semi-rotten and 15 dried) were randomly collected from five markets, namely; Abocho, Olowa, Etutekpe, Anyigba and Egume. The samples were properly preserved in nylon bags to prevent contamination. This was followed by extraction protocol established by the International Institute of tropical agriculture laboratory Ibadan (IITA, 2016). The extracts were analysed for aflatoxin B1, B2, G1 and G2 using Thin Layer Chromatography (TLC) with scanning densitometer, having detection limit of 0.05 ppb. The result obtained showed o.oo ppb for all the samples analysed. This is an indication that all samples analysed were free from aflatoxin contamination. This report may suggest that tomatoes sold within this area are safe for consumption.

Introduction

Mycotoxins are secondary metabolites of moulds that exert toxic effects on animals and humans [1]. Aflatoxins are poisonous secondary metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. The four major aflatoxins are B_1, B_2, G_1 and G_2 because of their prevalence in nature and toxicity [2]. Aflatoxins especially AFB_1 is a powerful carcinogen [3]. The toxins are also mutagenic and teratogenic [4]. The group of deadly mycotoxins contaminates nuts and oilseeds, cereals, roots and tubers, fruits, vegetables and animal feeds [2]. Humans can be exposed to aflatoxins by the periodic consumption of contaminated food, contributing to an increase in nutritional deficiencies, immunosuppresion and hepatocellular carcinoma [5]. The biosynthesis of aflatoxins as secondary metabolites, is strongly dependent on growth condition such as substrate composition or physical factor such as pH, water activity, temperature or modified atmospheres depending on the particular combination of external growth parameters [6].

The most important factors that help predict the occurrence of aflatoxins in food include wheather conditions (temperature and atmospheric humidity), agronomical practices and internal factors of the food chain [7].

The tomato originated from central and south America and is the second most important vegetable in economic importance and consumption in the world second to potatoes. Tomatoes are consumed in divers ways, including raw, as an ingredient in many dishes, sauces, salads and drinks [8].

Bioactive substances such as Carotenoids (lycopen, β-caroten and lytein) phenolics (Flavonoids, Phenolicacid and Tannins) and vitamins (B, C, E) are obtained by the natural consumption of fruits. These compounds positively affects health for having inflammatory and anticancer effects and preventing chronic heart diseases and hypertension [9]. Consumers in fresh fruits market choose fruits mainly following criteria related to the external appearances [10]. Colour, size, shape, and texure are the first attributes evaluated. Lycopene is the most abundant carotene in red tomato fruits [8]. The biosynthesis is associated with the change in fruit colour from green to red when chloroplast are transformed to chloro. Its abundance is associated with genetic control, fruit development time, water supply and climatic conditions. Lycopene biosynthesis can be inhibited if high temperature (Above 30°C) occurs in fruits due to a high incidence of solar radiation in the epicarp [10].

This study is therefore intended to evaluate aflatoxin contamination of tomatoes marketed within Dekina Local Government Area of Kogi state, Nigeria using TLC technique for detection.

Materials and Methods

Sample Collection

Forty-five (45) tomatoes samples consisting of (15 fresh, 15 semi-rotten and 15 dried) were randomly collected from five major markets, namely; Abocho, Olowa, Etutekpe, Anyigba and Egume in Dekina local government area of Kogi State, Nigeria. The samples were properly preserved in nylon bags to prevent further contamination before analysis. The samples were collected in the month of May, 2016 for analysis.

Extraction of Aflatoxins

The fresh and the semi-rotten tomato samples were first sundried for four days and further oven dried at 40°Cfor 20 hours to completely remove moisture. The dried samples were grinded using a coffee miller and the samples were thoroughly mixed. 20 grams of the sample was weighed and mixed with 100ml 80% methanol, it was then blended for three minutes. The blended mixture was poured into a 250ml pyrex conical flask and sealed with para film. The sample was shook using orbit shaker at 4 ×100 revolution per minute (r.p.m) for 30 minutes. The blended mixture was filtered into a clean conical flask using What man no. 1 quantitative filter paper, 185mm. The filtrate was poured into a separating flask and 40ml of 10% sodium chloride and 20ml of n-hexane was added. The flask was shaken vigorously for 1 minute by hand and allowed to separate. The bottom was drained into a 250ml conical flask and the remaining entity was discarded in the separating funnel. The filtrate was poured back into a separating funnel, 25 ml of dichloromethane was added to it and shaken vigorously and the flask was allowed to stand to allow separation into top and bottom phase. The extract was drained through a bed of 5g anhydrous sodium sulphate into a 150ml beaker. 10m of dichloromethane was added to the remaining mixture in the funnel, it was shook vigorouslyto effect separation. The bottom extract or bottom phase was drained through a bed of 10g anhydrous sodium sulphate into a 150ml beaker that contains the first extract. The extract was allowed to dry overnight in the fume hood and set for analysis [11].

TLC Analysis of Aflatoxin

1ml of dichloromethane was put in the tube containing the extract and thoroughly mixed using a vortex mixer. Four (4) microliter of the mixed extract was picked and spotted on a calibrated TLC plate according to the codes on the extract. On the TLC calibrated plate, we have 18 dots, when counted from the left the 6^{th} dot is the G standard and the 8^{th} dot is the I standard; they served as control to compare the extract or sample on the plate when it is inserted in the developing tank after spotting on each dot which are sixteen without those two standards. Small solution of aflatoxin radar was added to the developing tank and the spotted plate was inserted in it (2 plates maximum) facing upward. with the aid of the solution in the tank, the extract now travelled up the plate (6cm above) while the dots are 1.5cm apart. After travelling the distance of 6cm the sample was immediately removed and dried for some minutes, it was then carried to the UV box to view the level of aflatoxin on the plate.

The quantification of the aflatoxin was done using a scanner and it was quantified in ppb [11].

Results and Discussion

Table 1. *Concentration of aflatoxins B_1, B_2, G_1 and G_2 (ppb) in fresh tomatoes using Thin Layer chromatography with scanning densitometer.*

Sample name	Sample size	Aflatoxin Type	Aflatoxin concentration (ppb)	No. of +ve sample	No. of –ve sample
Fresh tomatoes	15	B1	0.00	-	15
	15	B2	0.00	-	15
	15	G1	0.00	-	15
	15	G2	0.00	-	15
Semi-rotten tomatoes	15	B1	0.00	-	15
	15	B2	0.00	-	15
	15	G1	0.00	-	15
	15	G2	0.00	-	15
Dried tomatoes	15	B1	0.00	-	15
	15	B2	0.00	-	15
	15	G1	0.00	-	15
	15	G2	0.00	-	15

Note:
a. Limit of detection=0.05 ppb
b. Zero means the aflatoxin level is below detection limit of the analytical method (1ppb)
c. Values are means of two subsamples of each sample

Discussion

The results in table 1 of this study shows no presence of aflatoxins in any of the 45 samples comprising of 15 fresh, 15 semi-rotten and 15 dried tomatoes collected from five major markets in Dekina local government of Kogi state in the month of May, 2016. The result is similar to the research work carried out between 1979 and 1982 by the public health laboratory in Milan, Italy, using thin layer chromatography, analysed 14 samples of tomato paste and 14 samples of canned tomatoes and found no aflatoxins (detection limit: 5-10 µg/kg). These same samples were free from from patulin and ochratoxin [12]. In another similar study also in Italy, no aflatoxins were detected in 70 tomato product commercially available; 40 of juices, 20 of pastes with 28% solids, and 10 of pastes with 36% solids (detection limit: 1 µg/kg), [13]. The report of [14] showed no aflatoxin detected in any of the 64 analyzed samples of tomato products. The finding of this research is also in line with the work of [15] who did not detect aflatoxins in dried tomato.

In contrast to the result of this research, is a survey of aflatoxin contamination between 2001 and 2002 in Nigeria, rotten tomatoes from 5 local markets were positive for aflatoxin contamination even after autoclave treatment at 121°C for 15 minutes [16]. [17], also detected some levels of aflatoxins in fresh and dried tomatoes; 6 of 25 fresh samples and 7 of 25 dried samples were positive to aflatoxins respectively

There are rare incidence of fresh tomatoes contamination by significant concentration of aflatoxin because fresh tomatoes contains polyphenols which to some extent inhibit the synthesis of aflatoxins, although the adequate inhibitory concentration of these compounds in tomatoes are yet to be determined [18].

The presence of a toxigenic fungus in foodstuff is not the only factor responsible for the production since fungal growth and aflatoxin production are the consequence of interactions among the fungus, the host, and the environment. Other factors like the extent of the infection and proper conditions for toxin production such as temperature, water content, medium composition, and the absence of antagonistic microorganisms are also important aspects that lead to detectable amounts of toxins [19]. The fungus infection can be avoided in the field by the maintenance of the good agricultural practices. Therefore, the fruit selection and storage under proper conditions in the processing stages are crucial in order to guarantee a final product free from toxin.

Another important aspect of the mycotoxin production in foodstuff is the presence or absence of compound that can inhibit the toxin synthesis.

Tomato mixed cropping and other post harvest mis-management which increases the chances of aflatoxin contamination in tomato fruits are not common among tomato farmers in Dekina local government. Early planting and harvesting habits are also exhibited among them.

Conclusion

The outcome of this research showed no aflatoxin contamination of tomatoes in all the samples considered. It is therefore safe to say that tomatoes sold in this area of study are safe for consumption with respect to aflatoxin contamination.

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Suleiman Mohammed Salisu

Suleiman Mohammed Salisu is an assistant lecturer at the Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria. He holds a B.Sc in Biochemistry from University of Ilorin, Nigeria, M.Sc in Biochemistry from Federal University of Technology Minna, Nigeria. He is currently seeking a PhD admission in some University both home and abroad. His research interests include; mycotoxin study and public health. His research work has being published in reputable journals and has attended annual scientific conference of Society for Experimental Biology of Nigeria. Email address: salisus570@gmail.com, Salmania82@yahoo.com.

Ojogbane Ele-Ojo

Ojogbane Ele-Ojo is a senior lecturer at the Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria. She holds a B.Sc in Biochemistry from University of Calabar, Cross river state, Nigeria, M.Sc in pharmacological Biochemistry, University of Nigeria Nsuka, Enugu State, Nigeria. And a PhD in pharmacological Biochemistry, University of Nigeria Nsuka, Enugu State, Nigeria. Her research interests pharmacological Biochemistry. His research work has been published in many international and national journals and has attended many international and national conferences. Email address: elgbane@yahoo.com

Omede Ameh

Omede Ameh is a lecturer II at the Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Nigeria. He holds a B.Sc in Biochemistry from Kogi State University, Anyigba, Nigeria, M.Sc in Biochemistry from Obafemi Awolowo University, Nigeria and a PhD in Medicine (cardiovascular science) from the University of Manchester, UK. His research interests include; drug discovery and heart failure. His research work has been published in reputable journals and has attended local and international conferences. Email address: omedeameh@gmail.com

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