GMP Journals
Journal of Contemporary Chemistry
Volume 1, Issue 1, P. 1 - 11, 2022
www.gmpjournals.com

Research Paper

Detection of Aflatoxins from Maize Samples Using Ferric Chloride

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Re-submitted on 28th September 2022

Published on 31st October 2022

ABSTRACT

Enzyme *naringinase* is a metabolite produced by a fungus called *Aspergillus flavus* and it changes colour from yellow to brick red when reacted with ferric chloride. Structurally, this enzyme is only two fewer carbon atoms compared to aflatoxins and it is produced by the same fungus that produces aflatoxins. Due to this structural relationship, the research borrowed the idea behind the colour change that occurs when enzyme *naringinase* reacts with ferric chloride to give a notable colour change. The current methods for detecting aflatoxins are expensive, time-consuming and some of them such as thin layer chromatography (TLC) are less sensitive and use carcinogenic reagents. The main aim of this research was to develop a fast, economical and sensitive assay for the detection of aflatoxin in maize. The isolation of aflatoxins from the maize sample was done through a direct method using 70% methanol. The screening of the presence of aflatoxin was done using an enzyme linked immunosorbent assay (ELISA) test. A chemical test was later conducted on aflatoxins using 1% ferric chloride to give a brick red colour. The concentration of this colour determined the quantity of aflatoxin. This study is relevant because aflatoxin is a killer toxicant and it also reduces the productivity of our livestock and crops. Therefore, there is a need for early detection of aflatoxin before it spreads to a wide range and causes further harm. Thus, using ferric chloride as a method for detecting aflatoxins is convenient because it is a rapid, simple and sensitive method compared to other methods such as thin-layer chromatography.

Key words: Aflatoxins, Thin Layer Chromatography, Optical Density, ELISA, Parts per billion.

1.0 Introduction

Aflatoxin is one of the most illustrious mycotoxins spawned by several species of aspergillus including *A. flavus* and *A. parasiticus* in a broad variety of agricultural

commodities including grains (maize), legumes and nuts (Wacoo *et al*, 2014). The main aflatoxin producing *A. flavus*, *A .parasiticus* and *A. nomius* can infect maize from pre-harvest stages in the stores. Species of *aspergillus* are almost ubiquitously present in soils of tropical areas (Piñeiro, 2014). Even though most species of *aspergillus* are not of much consequence in agriculture, some species are found in plant products, particularly oil-rich seeds. Contamination of seeds with highly poisonous aflatoxins results from the presence of toxigenic strains of four species of *aspergillus*: *A. flavus*, *A. parasiticus*, *A. nomius* and *A. bombycis*, each producing a combination of different types of aflatoxins (Piñeiro, 2014).

In Africa, aflatoxins have an impact on human and animal health and trade. Aflatoxins have been reported to be associated with the exacerbation of the energy malnutrition syndrome in children and vitamin A malnutrition in animals. In various animal models, in addition to being hepatotoxic, aflatoxin causes significant growth faltering and is strongly immune-suppressive at weaning (Gowda, 2013). Similar effects have been reported in human population in a few African countries such as Ghana and it has been recently shown that 99% of all children weaned from mother's milk to maize-based diets in Benin and Togo had aflatoxin in their blood, indicating ingestion of aflatoxin-contaminated food (Piñeiro, 2014).

In developing countries, the contamination of crops with aflatoxin leads not only to economic losses but also has a severe impact on human health. In Africa, a continent that relies on vulnerable crops such as groundnuts and maize as dietary staples, aflatoxin contamination causes major health problems (Bbosa *et al*., 2013). People in rural areas may have no option but to consume contaminated crops daily. This moderate, chronic intake of aflatoxin via food can lead to severe pathological conditions including liver cancer, immune system deficiency and impaired development of children (Bbosa *et al*, 2013). Malnutrition, a common condition in rural Africa, increases disease prevalence and further reduces the

ability of the human body to cope with aflatoxin exposure. Chronic aflatoxin poisoning reduces life expectancy.

Acute aflatoxin poisoning is caused by the ingestion of high levels of the toxin. Immediate consequences are severe liver damage, acute jaundice, and hepatitis, which may subsequently result in death (Brigitte Gedek Award, 2014). Although on a global basis, deaths from acute aflatoxins poisoning are rare, Kenya has experienced dramatic outbreaks of mycotoxin poisoning resulting in loss of lives. In 2004, an acute aflatoxicosis outbreak occurred in Machakos, Kenya resulting in 317 cases and 125 deaths, while cases of liver cancer have been linked to high levels of aflatoxins in the Lake Victoria basin (Probst *et al*., 2007). This study is relevant to be undertaken because aflatoxin is a killer toxicant and it also reduces the productivity of our livestock and crops. In the position we are as a country with a food shortage crisis, we cannot allow the spread of aflatoxin to uninfected livestock, crops and most importantly the citizens. Therefore, there is a need for early detection of aflatoxin before it spreads to a wide range and causes further harm. Thus, the objective of this study was to determine whether ferric chloride could be used to detect aflatoxins as this could be a rapid, simple and sensitive method compared to other methods such as thin-layer chromatography (TLC).

2.0 Materials and methods

2.1 Study site

Machakos County is in a semi-arid zone in the Eastern region at an elevation between 800- 1700m with an annual rainfall of between 300 and 600mm and a mean temperature of 24°c. Machakos County has two maize planting seasons, from March to May and from October to December.

2.2 Sample collection

One hundred (100g) grams of shelled maize was collected randomly in a sterile paper bag from the household storage facilities, sealed and stored at 4°c.

2.3 Experimental procedure

Extraction and detection of aflatoxin from maize directly using methanol as described by Wacoo *et al*., (2014). Fifty grams (50g) of maize was weighed and put in a blender jar. Five grams (5g) of sodium chloride was then added in the blender jar. Thereafter 200ml of 70% methanol was added in the blender. The mixture was blended at a high speed for 3 minutes. Filtration was then done through 18.5cm Whatman paper and 15ml filtrate was collected. One per cent (1%) of ferric chloride was added to the filtrate and a colour change from yellow to brick red was observed. A confirmatory test was done on glucose but no colour change was observed.

Enzyme linked immunosorbent assay (ELISA) procedure was done as described by Wacoo et al., (2014). Two hundred microliters (200µL) of the aflatoxin-HRP conjugate were dispensed into each mixing well. One hundred microliters (100µL) of each standard and sample to be tested were added to appropriate mixing well containing conjugate using a new pipette tip for each. One hundred microliters (100µL) of content from each mixing well was transferred to a corresponding Antibody coated micro-titer well. This was then incubated at room temperature for 15 minutes. The contents from micro-wells were then decanted into the sink and then the emptied micro-wells were washed for a total of five washes. The micro-wells were tapped on a layer of absorbent towels for drying purposes. One hundred microliters (100µL) of substrate reagent was added into each micro-well and incubated for 5 minutes. One hundred microliters (100µL) of the stop solution was then added. Afterwards the optical density of each micro-well was read using a model 680 micro-titer plate reader using a 450nm filter.

3.0 Results

In the results below, I found out that the intensity of a brick red colour change is directly proportional to the concentration of aflatoxins in the samples.

Figure 1. *(a) 20ppb which is the highest conc. (b) 5ppb which is the moderate conc. (c) maize sample(1ppb) the lowest conc. (d) water(control) (e) extracting solvent (methanol).*

Figure 1 shows the decrease of colour intensity from left to right as the concentration of aflotoxins in the samples decreases. The last two conical flasks (on the right) are water and the extracting solvent (70% methanol) respectively. None of them showed colour change ruling out that they may be the cause of colour change.

Figure 2. *Confirmatory test with glucose.*

Figure 2 shows a confirmatory test by reacting out ferric chloride and glucose. This was to rule out the possibility of glucose in the maize samples was the reason behind the colour change. The ferric chloride retained its yellow colour and therefore no colour change was observed.

Table 1. A table showing the optical densities and the aflatoxin concentrations of the standards and samples read by the microtiter plate reader

ID	Optical density	Concentration (ppb)
Standard 1	2.967	0.00
Standard 2	2.911	1.00
Standard 3	2.599	2.50
Standard 4	1.504	5.00
Standard 5	0.711	10.00
Standard 6	0.891	20.00
Sample 1	2.885	1.12
Sample 2	2.907	1.02
Sample 3	2.828	1.40
Sample 4	2.840	1.34
Sample 5	2.854	1.27
Sample 6	2.196	3.427
Sample 7; sample of interest	2.909	1.01
Sample 8	2.808	1.50

Table 1 shows the optical density and aflatoxin concentrations confirming the presence of aflatoxins in the maize samples using the ELISA method. Eight (8) maize samples were compared with six standards of aflatoxins of different concentrations (0ppb, 1ppb, 2.5ppb, 5ppb, 10ppb and 20ppb). The maize sample of interest was maize sample 13 but other maize samples were also used in order to balance the micro-wells for perfect reading by the microtitre plate reader. The

sample of interest (sample 7) showed 1.01 ppb concentration of aflatoxins which is very low.

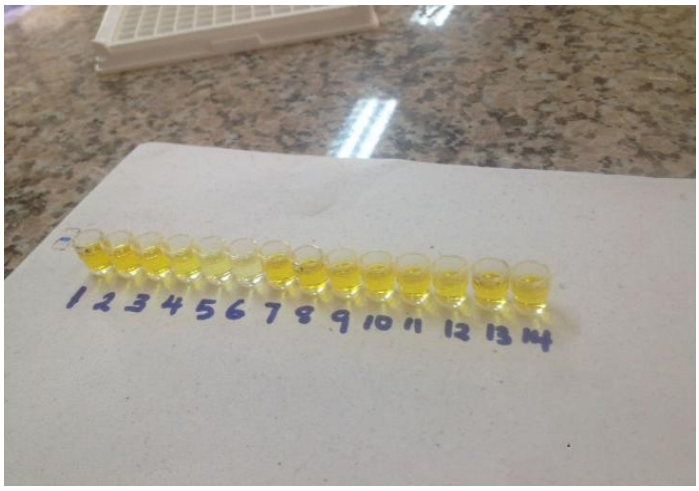

***Figure 3**. (1-6) standard concentrations of aflatoxins. (7-14) maize samples from different regions of the country. (13) Specific maize sample of interest.*

Figure 3 shows the confirmation of the presence of aflatoxins in the maize samples used by ELISA method. Maize samples were compared with six standards of aflatoxins of different concentrations (0ppb, 1ppb, 2.5ppb, 5ppb, 10ppb and 20ppb).

The maize sample of interest was maize sample 13 but other maize samples were also used in order to balance the micro-wells for perfect reading by the microtitre plate reader. The sample of interest (13) showed some low concentration of aflatoxins as shown in table 1.

4.0 Discussion

The structure of aflatoxins contains several unsaturated carbons and these sites are where the ferric chloride binds to form a complex that is brick red in colour. The more saturated the colour is the more the concentration of aflatoxin is in a particular sample, also a little bit of a brick red colour indicates that the level of aflatoxins in that particular sample is very low (Gowda, 2013).

The occurrence of colour change in the presence of a little amounts of aflatoxins indicates that using ferric chloride as an indicator to test for aflatoxin is a perfect method due to the aspect of sensitivity. In the results above, the concentration of 20 ppb showed a deep brick red colour when mixed with ferric chloride, at 5 ppb the colour was moderate and the colour was slightly less when ferric chloride was reacted with 1 ppb concentration of aflatoxins. Water did not show any colour change when reacted with ferric chloride thus this shows that ferric chloride is only sensitive to aflatoxins. That rule out that the extracting solvent (methanol) and glucose molecules found in maize could be the possible cause of colour change, ferric chloride was also reacted with these two and there was no notable colour change observed.

To confirm the presence of aflatoxins in the maize sample used, an ELISA test was carried out. These maize samples were compared with six standards of aflatoxins of different concentrations (0 ppb, 1 ppb, 2.5 ppb, 5 ppb, 10 ppb and 20 ppb). The maize sample of interest was maize sample 13 but other maize samples were also used to balance the micro-wells for perfect reading by the microtitre plate reader.

After the ELISA reaction, the resultant solutions were yellow in colour but some were more yellow compared to others. The intensity of the colour is directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxin in the sample or standard sample. Therefore, as the concentration of aflatoxins in the sample or standard increases, the intensity of the yellow colour decreases.

The micro-wells were measured optically by a micro-plate reader with an absorbance filter of 450 nm. The optical densities (OD) of the samples were compared to the OD's of the kit standards and an interpretive result is determined. These results show that as the OD increases as the concentration of the aflatoxins increases.

5.0 Conclusion

Ferric chloride has proven to be a very useful chemical in the detection of aflatoxins. This chemical is cheaply available, chemically stable and also it is a non-corrosive chemical. The important aspect of using ferric chloride for aflatoxin detection is its sensitivity since it can detect concentrations of less than 2 ppb. This is advantageous compared to methods such as TLC 5 ppb and also uses carcinogenic chemicals. I conclude by campaigning for the use of ferric chloride as an indicator for detecting aflatoxins both in the field and in the laboratory set up.

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Acknowledgement

I would like to give thanks to all mighty God for giving me life, good health and the ability to come up with new ideas. If it was not for the Lord I would not have reached this far in my education.

Secondly, I would like to sincerely pass my regards to my supervisor Mr. Joshua Gikonyo who has supported me by helping me find equipped laboratory to do my work and also by guiding me all through the entire project. I would also wish to thank the entire staff in the department of biochemistry and biotechnology at The Technical University of Kenya who have helped this project to be a success in one way or another.

I would also like to thank Mrs. Dorcas Mutunga in the department of Food and Drugs at the Government Chemist, Nairobi who provided me with chemicals and equipment and also guided me through all the laboratory work.

Lastly, I would like to thank my beautiful family especially my mum Jemimah Matika for the emotional and financial support all through the entire period of my project work, my classmates and friends for their moral support.

Financial support

This project was financed by the collaboration of The Technical University of Kenya and the Government Chemist, Nairobi.

Conflict of interest

No conflict of interest was reported

Ethical approval

This study did not work on any human or animal subjects.

Cite this paper: Mukhaye, E. (2022). Detection of Aflatoxins from Maize Samples Using Ferric Chloride. Journal of Contemporary Chemistry, Volume 1, Issue 1, P. 1 - 11.

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