

A REVIEW OF AFLATOXIN CONTAMINATION IN FOODS AND BEVERAGES IN UGANDA

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BU/UP/2018/3520

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE AND EDUCATION DEGREE OF BUSITEMA UNIVERSITY.

DECLARATION

l Okongo	lan	declares	that	this	resea	arch	review	is	original	work	and	has	not	been
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Approval

This dissertation has been done under my supervision and guidance.

Supervisor:

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Sign	08.05.22

Dedication

This dissertation is dedicated to my dear parents, my father Mr. Obotha Martin and my Mum Wadaga Pamela, my aunt Wadaga Phoebe Zewulence and the entire family of Mrs. Katono Jesca (JAJA) for their financial support. Special thanks go to HESFB- Chapter for their endless financial and mentor support all through my bachelor's academic journey.

Acknowledgement

I would like to thank the Almighty God for the gift of life He has blessed me with, had it not been Him, I would not succeed in my education. Thank you Oh Lord!

I wish to express my sincere gratitude to my supervisor Dr. Andima Moses and Chief Lab Technicians Dr. Kigozi Moses and madam Nakijoba Lydia for their advisory support, guidance, Counseling and Knowledge they offered towards the development of ideas throughout this piece of work. Special thanks are extended to further my family members: my father Mr. Obotha Martin and my Mum Wadaga Pamela, Aunt Wadaga Phoebe Zewulence and the entire family of Mrs. Katono Jesca (JAJA), Obuya, Okongo Samuel, Wapino Moses and my sponsors the HESFB- Chapter for their financial support during my time of study at university.

I further appreciate my beloved friends; Mukisa Ahamdah, Diiri James Emmanuel, Wandulu Ambrose, Nakiwala Olivia, all chemistry and Biology colleagues for their sincere commitment, advice and guidance during the research work and writing up of the dissertation.

May God reward them abundantly.

ABSTRACT

Mycotoxins are fungal secondary metabolites that if ingested can cause a variety of adverse effects on humans. Aflatoxins are a form of mycotoxins that are cancer genic compounds produced predominantly by certain strains of the Aspergillus genus. They have immunosuppressive, mutagenic, teratogenic and carcinogenic effects, especially on the liver.

Aflatoxin contamination of agricultural produce poses a considerable risk to human health. The infection of these crops especially barley, sorghum, and cassava for making beers and malwa respectively by aflatoxicogenic fungi and hence contamination with aflatoxin is generally higher.

AFB₁, AFG₂, AFG₁, AFG₂, AFM1 and AFM₂ are the most common types of aflatoxin. Several researches show that AFB₁ is the most common aflatoxin in the world from most commodities at a very much higher level. This indicates that in a lot of developing countries, people are at danger of aflatoxin contamination. Lack of awareness on aflatoxin contamination increases the risk of damage to human.

A variety of physical, chemical and biological methods have been developed for decontamination and control of aflatoxins from contaminated foods. This study review is aimed at reviewing the prevalence of aflatoxins in beers that has not been realized before and are manufactured from barley, and malwa from cassava through the process of fermentation which provides a suitable environment for aflatoxin growth upto the time of consumption.

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1.0 INTRODUCTION

1.1 background

Aflatoxin (AF) comes from a combination of "a" which stands for *Aspergillus* genus, "fla" which stands for species *flavus*, and toxin that stands for poison (R. Bhat, 2010). In this context, aflatoxins are poisonous secondary metabolites produced by *Aspergillus flavus*.

The discovery of aflatoxins dates back to 1960s in England. During this period, Over 100,000 turkeys, 20,000 ducklings, pheasants, chicks, and partridge poults were reported to have died from a fatal incident as noted by (Blount, 1961). The earlier history of the above disease (Turkey "X" disease) outburst in Great Britain was depicted in sufficient brass tacks by Blount (Blount, 1961). With time, a recognizable pattern of signs of the disease was reported in tamed animals outside Great Britain. And finally, the acting mold causing the problem, *Aspergillus flavus*, was isolated from a meal later, a compound that acts on the liver in ducklings in Uganda (K. Sargeant, 1961), and the toxicity noted in diverse animal species was repeated by Allcroft (Allcroft, 1964). The above as well was caused by a sequence of fluorescent compounds in a peanut meal that was given to the poults, imported from Brazil (Richard, 2008).

Chemically, Aflatoxins are highly oxygenated polysubstituted bicyclic aromatic

compounds, 1,2-benzopyrone or any of its derivatives with structures which slightly are different. There are six major aflatoxins commonly found in foods and include; aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) (Q.Wu, 2009), aflatoxin M1 (AFM1), and aflatoxin M2 (AFM2). The B-aflatoxins, usually the pentanone derivatives, show a greater blue fluorescence under ultraviolet light whereas the G-series that are six-membered lactones fluoresce yellow-green on thin-layer chromatography plates, hence the B and G appellation (Klich, 2003). AFB2 and AFG2 are dihydroxy derivatives of AFB1 and AFG1, and the rest of the (H. S. Chun, 2007) noted that AFs are not usually reported in the absence of AFB1. The M series are toxic metabolic derivatives of the B series that show blue-violet fluorescence and have been identified in the milk for animals that fed on feeds contaminated with aflatoxins (A. P. Wacoo, 2014), hence the appellation M (Klich, 2003). The subscripts 1 and 2 in AF nomenclature are designations for major and minor, respectively. The minor AFs have received description as mammalian biotransformation products of the major metabolites (S. Okoth, 2016).

Aspergillus flavus, A. nomius, A. parasiticus and A. tamarii are the main producers of aflatoxins (R. E. Black, 2013), that are commonly soil borne fungi, and are a primary cause of plant materials decomposition. Approximately 20 Aspergillus species were reported to yield AFs (N. Baranyi, 2013), although a physical examination of more novel with the most common ad position being to aflatoxigenic fungi proceeds (J. varga, 2015). Most of the species yield the B-type AFs passing through the polyketide pathway as difuranocoumarin derivatives though species related to A. parasiticus, A. nomius, A. toxicarius, A. bombycis, A. parvisclerotigenus, A. minisclerotigenes, and A. arachidicola are capable of additionally producing the G-type aflatoxins (J. varga J. F., 2009). AFM₁, AFM₂, AFB₂A, and AFG₂A have been isolated from cultures of A. flavus and A. parasiticus whereas AFGM₁, parasiticol, and aflatoxicol are mostly produced by A. flavus.

Aflatoxins have been detected in most Uganda foods and have a serious health problem attributed to liver cancer. Liver cancer has been increasingly recorded in the health sector attributed aflatoxin exposure to human lives. AF also lead to other health

consequences like acute aflatoxicosis, affects negatively protein synthesis and brings about blood clotting, increase in body weight and immunogenesis. 8.19 liver cancer cases have been reported per 100000 inhabitants annually in Africa. Of these cases, about 3700 are from Uganda. For individuals that are hepatitis B and C surface antigen carriers, the likelihood of them getting liver cancer is recorded as high as over 50%.

1.2 Problem statement

In Uganda, liver cancer has been increasingly being recorded in the health sector attributed aflatoxin exposure to human lives. AFs also lead to other health consequences like acute aflatoxicosis, affects negatively protein synthesis and brings about blood clotting, increase in body weight and immunogenesis.

8.19 liver cancer cases have been reported per 100000 inhabitants annually in Africa. Of these cases, about 3700 are from Uganda. For individuals that are hepatitis B and C surface antigen carriers, the likelihood of them getting liver cancer is recorded as high as over 50%.

Foods and beverages that are produced by the process of fermentation that proceeds up to the time of consumption, which provides favorable environment for aflatoxigenic fungal multiplication.

Aflatoxins have been detected in most Uganda foods but not yet in some like beers, meat blood and this study review will focus on levels of AFs in commonly consumed foods in Uganda.

1.3 RESEARCH OBJECTIVES

This study review is aimed at assessing AF levels in commonly consumed foods in Uganda, which include; peanuts/groundnuts, beans and cereals like; millet, maize, sorghum and cassava as well.

Specific objectives

- To assess AF levels in common foods like; peanuts/groundnuts, beans and cereals like; millet, maize, sorghum and cassava
- To compare the levels in the different commonly consumed foods.

1.4 JUSTIFICATION

Uganda is an agricultural country where approximately 60% of her people depend on farming as a source of income and food. Aflatoxins remain a persistent cause of illness and cause of suffering to people in the country, unprecedentedly reducing the nutritional and economic value of agricultural foods.

Aflatoxins cannot be readily removed from contaminated foods by detoxification. Therefore it is needed to develop the biological control methods that can ensure the crop safety by reducing the toxin content. This review is aimed at minimizing contamination through preharvest management, timely harvesting, and protection of crops from pest attack and country wide awareness creation. If not done, there will be continuous increase in the number of cancer cases reported in the country Uganda, which is a big risk to peoples' health. In addition, it will be very hard for Uganda to sell her agricultural food products to other countries due to unregulated levels of aflatoxins.

1.5 REVIEW RESEARCH METHODOLOGIES

The information used in this study review was systematically searched from a number of scientific journal articles published in English from Google scholar search engine. These papers were obtained by the use of a number of keywords. Some of the data was obtained from book section detailing quantification methods, advantages and disadvantages.

1.5.1 detection and quantification methods

In this section, data was obtained from various purely viewed and published articles in English from the Google scholar using the **Keywords**: "aflatoxin", "detection", "quantification methods", "advantages and disadvantages".

The link below was very instrumental with the use of the above keywords in obtaining the necessary articles with the required information.

https://scholar.google.com/scholar?hl=en&assdt=0%2C5&%q=aflatoxin+AND+foods+AND+Uganda&btnG=

This generated over 1000 articles that explain the different detection and quantification methods of aflatoxins with their advantages and disadvantages. Since the numbers of articles were so many, a criterion of selection was designed, where only articles published between 2006 to date were selected. This reduced the number of articles by almost 600 articles and the remaining articles were still too much to be compared as it could take much time. Another criterion therefore was designed and this depended on the number of citations for each article where the articles with 40 and more citations were selected. Basing on this, the best articles were selected and read by comparing till Lextracted the relevant desired information.

1.5.2 characterization

The information concerning the characterization of aflatoxins were obtained through the same way but using key wards of "aflatoxin", "foods", and "Uganda" and the criteria of selection remained the same as that of research methodology section.

1.6 REVIEW OF RESULTS AND DISCUSSION

1.6.1 RESULTS

As reflected in the tables 2, 3, 4, and 5 are the results that we obtained from various English scientific published articles up to date from Google scholar search engine. These papers were obtained by the use of a number of keywords. Some of the data was obtained from book section detailing detection, analysis and quantification methods of aflatoxins

The results were systematically searched from papers that were obtained with the help of the link <a href="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/scholar?hl=en&as-sdt=0%2C5&%g=aflatoxin+AND+foods+AND+Uganda&btnG="https://scholar.google.com/s

By using the keywords: "aflatoxins", "detection", "foods", "results" and "Uganda"

REFERENCES

- (PACA), P. f. (2017). Country-Led Aflatoxin and Food Safety Situation Analysis and Action Planning for Uganda. AddisAbaba, Ethiopia: Final Report, PACA, Af rican Union Commission.
-] A. Seetha, W. M. (2017). "Occurrence of aflatoxins and its management in diverse cropping systems of central Tanzania,. *Mycotoxin Research*, *33*, 323–331.
- A. J. A. Essers, C. E.-N. (1995). Reducing cassava toxicity by heap-fermentation in Uganda. International Journal of Food Sciencesand Nutrition, 46, 125-136.
- A. Lopez and M. A. Crawford. (1967.). Aflatoxin content of groundnuts sold for human consumption in Uganda. *The Lancet, 290,* 1351–1354.
- A. N. Kaaya, C. H. (2006). peanut aflatoxin levels on farms and in markets of uganda. *peanut science*, 68-75.
- A. P. Wacoo, D. W. (2014). Methods for detection of aflatoxins in agricultural food crops. *Journal of Applied Chemistry*, 15.
- A. P. Wacoo, D. W. (2014). methods of detection of aflatoxins in agricultural food crops. *journal of applied chemistry*, 15.
- A. P. Wacoo, D. W. (2018). feasibility of a novel on-site detection method for aflatoxin in maize flour from markets and selected housebholds in Kampala, Uganda. *Toxins*, *10*, 327.
- A. Seetha, W. M. (2017). Occurrence of aflatoxins and its management in diverse cropping systems of central Tanzania. *Mycotoxin Research, vol. 33, no. 4, pp. 323–331, 2017., 33,* 323-331.
- A. Wacoo, I. M. (2019). probiotic enrichment and reduction of aflatoxins in a traditional africa maize based fermented food. *Nutrients*, 265.
- Allcroft, R. (1964). Aspects of aflatoxicosis in farm animals. Cambridge University press.
- Blount, W. P. (1961). Turkey X disease. Journal of British Turkey Federation, 9, 52-61.
- D. K. Okello, M. B. (2010.). Overview of groundnuts research in Uganda: past, present and future. *African Journal of Biotechnology, 9*, 6448–6459.
- D. Kitya, G. B. (2019). aflatoxin levels in common foods of south western uganda: a risk factor to hepatocellular carcinoma. *european journal of cancer care, 19,* 516-521.

- Eboku, A. N. (2010). mould and aflatoxin contamination of dried cassava chips in eastern uganda: association with traditional processing and storage practices. *journal of biological sciences, 10,* 718-729.
- F. Wu, C. N. (2011). *The Health Economics of Aflatoxin: Global Burden of Disease,.* Washington, DC,, USA: Interna- tional Food Policy Research Institute.
- G. Asiki, J. s. (2014). a pilot study to evaluate aflatoxin exposure in a rural ugandan population. *tropical medicine and international health, 19,* 592-599.
- G. Bigirwa, G. S. (2006). fungal microflora causing ear rots in uganda and associated aflatoxins. *journal of biological sciences, 6,* 540-546.
- H. K. Taligoola, M. A. (2010). Toxigenic fungi and aflatoxins associated with marketed rice grains in Uganda. *Journal of Basic and Applied Mycology, 1*, 45-52.
- H. S. Chun, H. J.-B.-H. (2007). determination of aflatoxin levels nuts and their products consumed in south Korea. *food chemistry, 102,* 385-391.
- Herzallah, S. M. (2009). Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chemistry*, 1141–1146.
- J. Osuret, G. M. (2016). aflatoxin food contamination of selected staple foods sold for human consumption in kampala markets, uganda. *journal of biological sciences, 16,* 1-5.
- J. varga, J. F. (2009). A reappraisal of fungi producing aflatoxins. *world mycotoxin journal*, 263-277.
- J. varga, N. B. (2015). mycotoxin producers in the aspergillus genus: an update. *acta biologica szegediensis*, *59*, 151-167.
- J.D.Thrasher. (2012). AflatoxicosisinAnimals. Aflatoxins and Health.
- Jewers, J. K. (2015). Mycotoxins and their Effect on Poultry Pro- duction,. *Tropical Development and Research Institute (TDRI), Iondon, UK*.
- K. Sargeant, A. s. (1961). Toxicity associated with certain samples of groundnuts (Vol. 192).
- Kensley, J. D. (2005). role of metabolism and viruses in aflatoxin induced liver cancer. *toxicology and applied pharmacology*, 131-137.
- Klich, J. W. (2003). mycotoxins. microbiology reviews, 16, 497-516.
- M. A. Ismail, H. T. (2011). toxigenic mycobiota associated with baby foods locally produced in uganda with special reference to aflatoxins. *journal of basic and applied mycology, 2*, 55-67.

- M. S. Kanalg, p. N. (2015). longitudinal evaluation of aflatoxin exposure in two cohorts in southwestern uganda. *food additives and contaminants, 32*, 1322-1330.
- M.E.Alpert, M. G. (1971). Association between aflatoxin content of food and hepatoma frequency in Uganda. *Cancer*, *28*, 253–260.
- Monitor, D. (2019, April). New Technology to Clean Aflatoxins from Grains. http://mobile.monitor.co.ug/Business/Business/New-technology-to-clean-aflatoxins-from-grains/ 2471012-5062312-format-xhtml-s6h8wgz/index.html.
- N. Baranyi, S. K. (2013). current trends in aflatoxin research. acta biologica szegediensis, 95-107.
- News, P. (2018). "Quarterly newsletter of the partnership for aflatoxin control in Africa-African Union. *5*.
- Omara, T. (2019). Aflatoxigenic contamination of white maize from some selected ugandan districts. 7.
- P. Fandohan, D. Z. (2005). Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *International Journal of Food Microbiology, 98*, 249–259.
- P. Khlangwiset, G. S. (2011). Aflatoxins and growthimpairment:areview. *CriticalReviewsinToxicology, 41,* 740-755.
- Q.Wu, A. J. (2009). Biological degredation of aflatoxins (Vol. 41). drug metabolism reviews.
- R. Bhat, R. R. (2010). mycotoxins in food and feed (Vol. 9).
- R. E. Black, C. G. (2013). maternal and child undernutrition and overweight in low-income and middle-income countries. *the lancet, 382,* 427-451.
- R. Echodu, H. E. (2018). Is nodding syndrome in Northern Uganda linked to consumption of mycotoxin contaminated food grains. *BMC Research notes, 11,* 678.
- R. Kajobe, E. K. (2016). The status of honeybee pests in uganda. *Bulletin of Animal health and production in africa*, 105-117.
- R. M. Sabran, M. R. (2012). "Socio- demographic and socio-economic determinants of adults' knowledge on fungal and aflatoxin contamination in the diets. *Asian Pacific Journal of Tropical Biomedicine, 2*, S1835–S1841.
- Richard, J. L. (2008). Discovery of aflatoxins and significant historical features (Vol. 27).
- S. A. Baluka, D. S. (2017). mycotoxins and metallic element concentrations in peanut products sold in uganda markets. *cogent food and agriculture, 3*.

- S. Byakika, I. M. (2019). potential application of lactic acid staeters in the reduction of aflatoxin conatamination in fermented sorghum-milliet beverages. *international journal of food contamination, 6*.
- S. Muzoora, M. K. (2017). status of aflatoxin levels in groundnuts. *the pan african medical journal*, 11.
- S. Okoth. (2016). *improving the evidence base on aflatoxin contamination and exposure.*Wageningen: CTA working paper 16/13.
- T. Omara, S. N. (2018). Comparative evaluation of the microbial safety of boiled locally vended ready-to-drink, HTSTpasteurized and UHT bovine milk sold in Nakawa division of Metropolitan Kampala. *International Journal of Food Science and Nu- trition, 3*, 185–189.
- T. W. Kensler, B. D. (2011). a 50-year ordysse of mechanistic and translational toxicology. *toxicological sciences*, *120*, S28-S48.
- Tanaka, M. T. (2008). Determination of Aflatoxins in Food by LC-MS/MS. Agilent Technologies.
- Timothy Omara, W. N. (2020). Aflatoxins in Uganda: An Encyclopedic Review of the Etiology, Epidemiology, Detection, Quantification, Exposure Assessment, Reduction, and Control. *International Journal of Microbiology, 2020*, 18.
- Villers, P. (2014). Aflatoxins and safe storage. Frontiers in Microbiology, 5, 1-6.
- Vision, N. (2016, December 30th). EAGC Gives Equipment to Detect Aflatoxins. http://googleweblight.com/i?u�https://www.newvision.co.ug/new_vision/news/1443062/eagc-equipment-detect-aflatoxins&hl�en-UG.
- W. J. William, T. D. (2003). "Human aflatoxicoses in developing countries a review of toxicology exposure, potential health consequences and interventions. *American Journal of Clinical Nutrition*, 1106–1122.
- Warren, N. A. (2005). A review of past and present research on aflatoxin in Uganda. *African Journal of Food, Agriculture and Nutritional Development, 5,* 1−18.
- Williams, M. K. (1968). Hepatoma and groundnuts in the West Nile districts of Uganda. *Yale Journal of Biology and Medicine, 41*, 69–78.
- Yourtee, T. K. (1990). Aflatoxigenic Aspergilli in foods and feeds in Uganda. *Journal of Food Quality, 13,* 97–107.