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Occurrence and factors associated with aflatoxin contamination of raw peanuts from Lusaka district's markets, Zambia

N.F. Bumbangi [a, b, *], J.B. Muma [b], K. Choongo [c], M. Mukanga [d], M.R. Velu [b], F. Veldman [e], A. Hatloy [f], M.A. Mapatano [a]

[a] Department of Nutrition, School of Public Health, Faculty of Medicine, University of Kinshasa, PO Box 11850, Kinshasa I, Democratic Republic of the Congo
[b] Department of Disease Control, School of Veterinary Medicine, University of Zambia, PO Box 32379, Lusaka, Zambia
[c] Department of Biomedical Science, School of Veterinary Medicine, University of Zambia, PO Box 32379, Lusaka, Zambia
[d] Plant Protection and Quarantine, Zambia Agriculture Research Institute, P/Bag 7, Chilanga, Zambia
[e] University of Kwazulu Natal, South Africa
[f] Fafo, Box 2947 Toyen, N-0608 Oslo, Norway

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ABSTRACT

Peanuts, one of the most susceptible crops to aflatoxin (AF) contamination, are widely produced and consumed in Zambia. This cross-sectional study was designed to determine the levels of AFs in raw peanuts sold in Lusaka district's markets as well as identify factors associated with increased AF presence. Raw peanut samples were collected from open markets and supermarkets and analyzed for aflatoxin contamination using high performance liquid chromatography (HPLC). A questionnaire was also administered to the peanut vendors to investigate factors contributing to increased levels of AFs in peanuts. Of the 92 samples, 51 (55.4%; 95% CI: 44.9–65.4) tested positive for presence of AFs. The overall median and geometric mean ± standard deviation (SD) concentration for AF were 0.23 ppb (range: 0.014 −48.67 ppb) and 0.43 ± 9.77 ppb, respectively. The association between market types and presence of AFs was not statistically significant (Pearson $X^2 = 0.0587$, $p = 0.809$). Of 51 samples that tested positive to AF, 6.5% and 12% were above the maximum permissible limits (MPLs) set by the Codex Alimentarius Commission and European Union standards, respectively. There was a significant difference in the levels of AF between *Chalimbana* and *Kadononga* ($p<0.0001$), and also *Chalimbana* and *Makulu red* ($p<0.0001$). *Chalimbana* was the most at risk of AF contamination, when compared to other peanut varieties. The high level of AFs in raw peanuts from both supermarkets and open markets samples constitutes a health hazard for the population of Lusaka district. Therefore, intervention strategies that reduce the levels of AF contamination in peanuts should be given priority.

1. Introduction

Aflatoxins (AFs) are toxic metabolites of fungi (*Aspergillus* sp) that constitute one of the major food safety challenges (Unnevehr & Grace, 2013). Aflatoxins contaminate a large fraction of the world's food and feed commodities (Strosnider et al., 2006). Maize, peanut and cottonseed are the major crops affected by AFs (Cotty, Probst, & Jaime-Garcia, 2008; Kpodo, Thrane, & Hald, 2000). Human exposure to AF may primarily occur through contaminated intake (IARC., 2002), and secondarily from exposure to air and dust containing toxins released during the handling of contaminated products (Sorenson, Jones, Simpson, & Davidson, 1984).

Several harmful effects of AFs in both humans and animals have been described. These include liver cirrhosis, liver cancer, immune-system suppression, growth retardation for children and even death (Azziz-Baumgartner et al., 2005; Gong et al., 2004; Wild & Turner, 2002; Williams et al., 2004). Due to its hepatocarcinogenic effect, the International Agency for Research on Cancer (IARC) has classified aflatoxin B1 (AFB1) as a group 1 carcinogenic agent to humans (IARC., 2002).

In addition to their effects on human and also animal health, AFs constitute an economic burden. In fact, aflatoxin contamination of various agricultural products causes enormous losses to both

* Corresponding author. Department of Nutrition, School of Public Health, Faculty of Medicine, University of Kinshasa, PO Box 11850, Kinshasa I, Democratic Republic of the Congo.
E-mail address: bnflavien@gmail.com (N.F. Bumbangi).

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farmers (loss of livelihood) and the country through export bans which introduces additional cost in the treatment or rejection of banned products (Wu, Narrod, Tiongco, & Liu, 2011). Furthermore, the most susceptible crops to AF contamination (maize and groundnuts) are staple foods in most African communities (Wu and Khlangwiset, 2010). Therefore, any hazard occurring in these products is likely to affect a large population hence increasing poverty and food insecurity.

Since it is difficult to achieve zero tolerance with AF contamination in commodities, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommends that dietary exposure to AFs should be minimized as much as possible to prevent the risk of cancer (JECFA, 2008). Thus, legal tolerance limits based on scientific evidence obtained from risk assessment in different countries have been set for AFB1 and total aflatoxin (AF) in foods that are destined for human consumption (Wu, Stacy, & Kensler, 2013). The limits vary between 4 and 20 parts per billion (ppb) (Wu et al., 2013). The Codex Alimentarius Commission (CAC), Joint FAO/WHO Food Standards Program adopted levels of 15 ppb as maximum permissible limits (MPLs) for AF in unprocessed peanuts and tree nuts, and 10 ppb in ready-to-eat tree nuts (CAC, 2014). The European Union (EU) has the most stringent standards of AFs in the world with a limit of 4 ppb for AF (Wu et al., 2013). In most African countries aflatoxin is largely unregulated. In 2013, aflatoxin regulations were present in ten African countries (Wu et al., 2013). Zambia is one of the African countries that does not have its own regulation on AF but rely solely on the Codex Alimentarius Standards despite the fact that most of the staple diets in the country (maize, peanuts, and their products) (JAICAF, 2008; Sitko et al., 2011) are susceptible to AF contamination.

In Zambia, peanut production is high and contributes significantly to the national economy (Sitko et al., 2011). However, little is known about the magnitude of AF contamination in Zambian peanuts. Furthermore, there is currently no study in Zambia estimating the levels of AFs in peanuts sold at different markets. This study was carried out to measure the levels of AFs in raw peanuts sold in Lusaka district's markets as well as identify factors associated with increased AF presence in the Zambian peanut crops.

2. Materials and method

2.1. Survey and sample collection

A cross-sectional study design was conducted in Lusaka district's markets for a period of one (1) month, March 2015. Lusaka district was purposively selected because its markets receive agricultural products from all provinces of the country, other African countries and the world.

Market selling points for peanuts were categorised into open markets and supermarkets. An open market was defined as a market not housed in a building, where foodstuffs are sold exposed in the open air and spread on shelves or the ground; while a supermarket was defined as a market housed in a closed building with modernised facilities, i.e. shopping mall.

A list of open markets ($n = 57$) and supermarkets ($n = 12$) in Lusaka district formed the sampling frame of the study. Assuming that AFs in peanuts in Zambia occurred at 80% prevalence; and that we wanted to be 95% confident in estimating the true prevalence while allowing only 5% estimation error, the sample size was estimated using the formula for simple random sampling (Lwanga & Lemeshow, 1991).

Based on the above assumptions and after adjusting for fine population, a total number of 32 markets formed part of the study. Using a proportional stratified random sampling, a total of 26 open markets and 6 supermarkets were included in the study. Within each stratum (type of market), simple random sampling was done to obtain the required number of markets ensuring that all the seven constituencies of Lusaka district were represented. From each open market, at least 3 vendors were randomly sampled or 10% of them if the number was large. From each selected vendor, 500 g of raw peanuts samples were purchased. Similarly, from each supermarket, at least 500 g of raw peanuts of each variety were purchased. Further, a questionnaire was administered to those vendors to collect information on factors suspected to explain the occurrence of AFs in these products. Thus, 92 raw peanut samples were purchased with 73 from the open markets and 19 from the supermarkets.

2.2. Aflatoxins determination

Samples were analyzed in the chemistry laboratory at Zambian Agriculture Research Institute (ZARI) using AflaTest® test kit with HPLC method certified by the AOAC® Official Methods Program, as official method 991.31 applicable for the determination of aflatoxin B1, B2, G1 and G2 both by fluorometry and HPLC analysis in corn, peanuts and peanut butter.

Acetonitrile and methanol were purchased from Sigma–Aldrich® (Germany). For high-performance liquid chromatography, HPLC-grade reagents were used. Aflatoxin B1, B2, G1 and G2 standards were purchased from Trilogy Analytical Laboratory (USA) (Lot 120316–090, Total concentration AF: 5.0 μg/ml, Total aflatoxin B1, B2, G1, G2: 4/1/4/1). The concentration was determined according to AOAC International Official Methods of Analysis. An immunoaffinity column (IAC), the AflaTest® column (Vicam, Watertown, MA, USA), was used for cleaning the samples.

In order to minimize the sub-sampling error in AFs analysis, all the samples were ground using a domestic grinder (Jura-CAPRESSO INC, Model N°503, China) and 25 g of each ground sample with 5 g NaCl were weighed and mixed for analysis.

The mixture was placed in a blender jar for extraction using 125 ml of methanol: water (70:30). The solution was blended at high speed for 2 min and then filtered using fluted filter paper (Whatman No.4). After filtering, the extract was diluted with 30 ml of purified water before being filtered through a glass microfiber filter into a clean vessel.

AflaTest® immune-affinity columns (IACs) were used to clean up the samples. Fifteen milliliters of the filtrate diluted extract was passed through the AflaTest® IAC at a rate of about 1–2 drops/second until air came through column. Then, the column was washed twice with 10 ml of purified water at a rate of about 2 drops/second; and the glass cuvette (VICAM part # 34000) was placed under AflaTest® IAC and 1.0 ml HPLC grade methanol was added into glass syringe barrel. Finally, AflaTest® IAC was eluted at a rate of 1 drop/second by passing the methanol through the column and all of the sample eluate (1.0 ml) was collected in a glass cuvette. An additional 1.0 ml of purified water was poured to eluate and analyzed by HPLC.

Reverse-phase HPLC was used to quantify AFs along with fluorescence detector followed by post column derivatization (PCD) involving bromination using a water HPLC system (pump 1525; fluorescence detector 2475; analytical column Nova-pack-C18 250 × 4.6 mm: 5 μm). Kobra cell was used and bromide added to the mobile phase to achieve PCD. Fifty microliter of diluted AF eluate was then injected into HPLC. The mobile phase included water, methanol, and acetonitrile mixture with the 600:300:200 (V/V/V) ratio. A sample was considered as positive to AF if at least one of the four types was positively observed on HPLC chromatogram reading.

The limit of detection using the protocol described above is 0.10 ppb for total aflatoxin and 0.05 ppb for B1, 0.03 ppb for B2,