



Prevalence of aflatoxin and fumonisins ($B_1 + B_2$) in maize consumed in rural Malawi



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ABSTRACT

A study was carried out to assess levels of contamination of aflatoxins and fumonisins ($B_1 + B_2$) in maize produced, stored and consumed in rural households in Malawi. A total of 9 districts were selected across the country representing 3 districts from each of the Northern, Central and Southern regions respectively. Households were selected at random in each district where 10 maize samples were collected for laboratory analysis. Aflatoxins and fumonisins were analyzed using a single step lateral flow immunochromatographic assay based on a competitive immunoassay format. The detection limit for aflatoxins was 2 $\mu\text{g/kg}$ with a quantitation range of 2–150 $\mu\text{g/kg}$ and that for fumonisins was 1 mg/kg with a quantitation range of 1–7 mg/kg . It was found that samples in the Southern region were highly contaminated, with the Chikhwawa district having high levels of both aflatoxins and fumonisins in maize. The Northern region had the least contamination. The maximum detected amount of aflatoxins was 140 $\mu\text{g/kg}$. The maximum detected amounts of fumonisins was 7 mg/kg . About 20% of maize samples exceeded the tolerable maximum limit for aflatoxins in Malawi. Aflatoxins and fumonisins were found to co-occur with contamination levels exceeding 100 $\mu\text{g/kg}$ for both aflatoxins and fumonisins.

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1. Introduction

Mycotoxigenesis in sub-Saharan Africa is due mainly to aflatoxin contamination. About 250,000 hepatocellular carcinoma-related deaths occur annually in parts of sub-Saharan Africa due to aflatoxin ingestion alone [30]. Up until the mid-1990's reports of acute aflatoxin poisonings, approximately 25% of which result in deaths has been reported [30].

No serious mycotoxigenesis outbreak has been reported so far in Malawi, but the climatic conditions, outbreaks of mycotoxigenesis in neighboring countries and knowledge of pre- and post-harvest practices strongly suggest that Malawians are consuming mycotoxin-contaminated foods. This research, therefore, was aimed at assessing the extent of mycotoxin contamination in maize consumed in Malawi.

Malawi is a country in southern Africa with a population of about 17 million people. The backbone of Malawi's economy is agriculture, which employs about 90% of the population. Agriculture contributes more than 35% of the country's gross domestic product (GDP) and accounts for almost 85% of the export earnings [18].

Maize in Malawi is the most important staple food crop; it is grown by 97% of farming households and accounts for 60% of total food consumption. It is cultivated on more than 70% of the total arable land and contributes significantly to diets of more than 80% of the population, with per capita consumption of 182 kg per year [16].

Over half of Malawi's farming households operate below subsistence. Because of low productivity and small farm size, only 20% of maize farmers produce surplus and sell their product [6]. This means that about 80% of these farmers are not able to produce enough maize for their own home consumption. Most farmers will sell the best quality maize that they have. As a result what is left as food for family consumption is frequently grain of poor quality, some of which may be contaminated by mycotoxins, leaving this population at a health risk.

Food safety with its relationship to food quality in the developing countries of Africa is an issue which frequently must be balanced by issues of food security with an emphasis on sufficiency of supply [25].

The lack of an effective regulatory and enforcement framework coupled with a lack of consumer awareness and understanding of the effect of molds and mycotoxins on human health combine to increase risk to human health. Currently in Malawi there are insufficient data on the extent of fumonisin prevalence and there is only limited data regarding aflatoxin contamination in maize. Aflatox-

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ins are regulated in Malawi, but this only applies to maize or any farm produce meant for export or for those products meant for super markets. Fumonisin on the other hand are not regulated currently in Malawi; however, there is as much risk associated with consuming maize contaminated with fumonisin as is the effect of consuming maize contaminated with aflatoxins [20,32]. It has been shown in Tanzania that aflatoxins coexist with fumonisins in maize [14], and there is also evidence suggesting that aflatoxins act synergistically with fumonisins [20] putting consumers at more risk from their combined effects. When animals or humans consume foods contaminated with aflatoxins, AFB₁ is metabolized in the liver leading to formation of highly reactive chemical intermediates. The binding of these intermediates to DNA results in the disruption of transcription and in abnormal cell proliferation, leading to mutagenesis and carcinogenesis [10,12,24]. Consumption of moldy maize containing fumonisin B₁ has been associated with an outbreak of abdominal pain and diarrhea in India [2]. Pathogenic effects due to fumonisin ingestion in animals include leukoencephalomalacia, pulmonary edema, hepatotoxicity, hepatocarcinogenicity and nephrotoxicity [23]. Consumption of contaminated maize has been associated with an elevated risk of human esophageal cancer in the Transkei region in South Africa and China [32]. It has been shown that culture material of *Fusarium verticillioides* was hepatocarcinogenic in rats, exhibiting both initiating and promoting effects. FB₁ was subsequently shown to be a liver cancer promoter in a diethyl nitrosamine-initiated rat model. Fumonisin, in particular FB₁ are prototypic inhibitors of cellular sphingosine (sphinganine) *N*-acetyltransferase. Inhibition of this enzyme is followed by an accumulation of sphinganine and sometimes also sphingosine and a depletion of complex sphingolipids in eukaryotic cells. The beginning and progression of diseases associated with FB₁ have a close relationship to the disruption of sphingolipid metabolism [17,21,29]. This leads to impairment of cell cycle regulation and cellular differentiation. It also results in oxidative stress as well as apoptosis and necrosis [11]. Altered apoptosis and mitosis is thought to contribute to carcinogenesis through an altered balance of cell death and replication [31].

Approximately 25% of the world's food crops are affected each year by mycotoxins [4] with aflatoxin and fumonisin contamination being of particular importance. Most African countries are lagging behind industrialized countries in pre- and post-harvest practices that would minimize mycotoxin consumption. Donating ("dumping") mycotoxin contaminated food products and the introduction of contaminated commodities into the human food chain during acute and chronic food shortage due to drought, political and economic instability also contribute to the problem.

Aflatoxins are produced by the fungi *Aspergillus parasiticus* and *Aspergillus flavus* as secondary metabolites when the temperatures are between 24°C and 35°C. They form in many commodities in conditions of excess moisture during harvest and storage. Aflatoxins are considered by the United States Food and Drug Administration (USFDA) to be unavoidable contaminants of foods.

Among the aflatoxins and their metabolites, only AFB₁, AFB₂, AFG₁ and AFG₂ have been found as natural contaminants in agricultural products. They cause mycotoxicosis in poultry and mammals. Acute aflatoxicoses have been reported in humans in Taiwan, Canada, Uganda, Germany, India and Kenya [3,27,8].

AFB₁ ingestion by humans is becoming increasingly important as new results from laboratory animal studies and epidemiological studies are reported. Chronic aflatoxicosis with high incidence of primary liver cancer has been reported in Uganda, Thailand, Kenya, Mozambique and China [3,27]. Aflatoxin ingestion impaired child growth in Benin and Togo [9]. Despite of nearly 50 years of research, the extent of the global exposure to this carcinogen is still poorly documented, hampering estimation of the associated disease burden. Currently, the World Health Organization does not recognize

mycotoxins as a disease burden. Using aflatoxin biomarkers, it has been shown that aflatoxins cross the placental barrier as revealed by the presence of aflatoxin albumin adducts in cord blood samples [31]. In West Africa, this exposure has been shown to continue in infancy and once children are weaned, they have a similar high prevalence and level of exposure as observed in adults [31]. The regulatory limit for aflatoxins in Malawi is 3 µg/Kg but which is in the process of revision to match the limit in the Common Market for Eastern and Southern Africa (COMESA) harmonised standard.

Fumonisin, like aflatoxins, are a group of toxic metabolites produced by the molds *Fusarium verticillioides*, *F. proliferatum* and *F. nygamai* with *Fusarium verticillioides* being the predominant contaminant in food and feeds, (Michael and Wyatt, 1993). Fumonisin were first isolated in 1988 and consist of a long hydroxylated hydrocarbon chain with added tricarboxylic acid, methyl, and amino groups. They are polyols with a long chain (20 carbons) esterified in the C14 and C15 with two groups of tricarboxylic acids. Fumonisin B₁ (FB₁), Fumonisin B₂ (FB₂) and Fumonisin B₃ (FB₃) are the major naturally occurring fumonisins. However, Fumonisin A₁ and A₂ (FA₁ & FA₂) also occur naturally [23]. In 1993, the International Agency for Research in Carcinogenesis, (IARC) classified fumonisins as Group 2B compounds – "probably carcinogenic for humans" [13]. Fumonisin contamination of maize occurs in many parts of the world with reported levels greater than 100 mg/Kg in some regions. Fumonisin contamination of agricultural produce is dependent on geographical region, season and the conditions under which the particular grain is grown, harvested and stored. Grain grown in tropical and subtropical regions is more prone to fumonisin contamination due to the relatively long and warm growing season Michael and Wyatt, 1993. Contamination of corn with high levels of fumonisin has been reported in Tanzania, South Africa, United States and China [14,7,8,26].

Fumonisin B₁ is considered the most prevalent and most toxic derivative within the group of fumonisins [31]. Contamination of cereals with the fungus *Fusarium moniliforme*, a common contaminant of corn throughout the world, has been associated with several human and animal diseases. Consumption of moldy maize containing fumonisin B₁ has been associated with an outbreak of abdominal pain and diarrhea in India [2]. Pathogenic effects due to fumonisin ingestion in animals include leukoencephalomalacia, pulmonary edema, hepatotoxicity, hepatocarcinogenicity and nephrotoxicity [23]. Consumption of contaminated maize has been associated with an elevated risk of human esophageal cancer in the Transkei region in South Africa and China [32]. The regulatory limit for fumonisins in the United States is 2–4 mg/Kg (2 to 4 ppm) in foods meant for direct human consumption [28] and in the European Union it is 4 mg/Kg (4 ppm) in foods meant for further processing and 1 mg/Kg (1 ppm) in foods meant for direct human consumption, (European Union 1881/2006). The maximum tolerable daily intake limit as set by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) is 2 µg/kg body weight/day for FB₁, FB₂ and FB₃ alone or combined.

The regulatory limit by Codex Alimentarius is 4 mg/kg (4 ppm) for fumonisins B₁ + B₂ in unprocessed corn [5]. No regulatory limits have been set in Malawi for fumonisins.

2. Materials and methods

2.1. Sampling plan for maize

Maize is grown and consumed throughout Malawi and therefore a country-wide sampling plan was established. Malawi is divided politically into Northern, Central, and Southern Regions. Each region is divided into districts and three districts from each region were randomly chosen for sampling. The districts selected

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