

Prevalence of Aflatoxins in Smoked-Dried and Fresh Fish in Zambia

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Abstract

The food contamination is a critical public health concern at the global level. The aflatoxins are considerable food contaminants and health menace to a sizable world population. Aflatoxins originate from fungi as their toxic secondary metabolites. This study aimed to probe the contamination level of aflatoxins in smoked-dried fish and the extent of the threat it might pose on human health. The study considered five of the regularly consumed species of smoked-dried fish. The study also considered two species of fresh fish. The investigation was carried out using VICAM Series-4EX Fluorometer. The Buka fish sample had the lowest concentration of 1.3 ppb for total aflatoxins, and Bream fish was analyzed to have the highest as 3.84 ppb. As such, the total aflatoxin concentration in this study was found to be between 1.3 and 3.84 ppb. These concentrations can be considered to be a matter of concern. Prolonged intake of the aflatoxins in this range may result in a health hazard to humans. As expected, there was no trace of aflatoxins detected in fresh fish samples.

Keywords

Aflatoxins, Mycotoxins, Fluorometer

1. Introduction

The mycotoxins contamination of food merits serious consideration because of their adverse effect on human or animal health. These are quite stable molecules, and once they sneak in the foodstuff, it is difficult to remove [1]. The factors which affect the susceptibility of foodstuff to mycotoxin contamination include climatic conditions, storage standards, and harvesting mechanism [2]. The studies have established that there are interdependent physical, chemical, and bio-

logical factors that influence the production of mycotoxins [3]. There has been an extensive study on the devastating effects of mycotoxins on human or animal health [4] [5].

The mould fungi are known to generate aflatoxins, which are complex foodstuff contaminants. There are more than one hundred mycotoxins, which have already been identified. However, six significant aflatoxins merit special mention. These are B₁, B₂, G₁, G₂, M₁, and M₂ [3]. The aflatoxin M₁ and M₂ are hydroxylated forms of AFB₁ and AFB₂ present in milk [6] [7]. The production of several aflatoxins from different moulds has been reported [8]. The species producing the majority of these mycotoxins belong to three fungi genera, namely *Aspergillus*, *Penicillium*, and *Fusarium* [9] [10]. The metabolism of diverse fungi species related to the *Aspergillus* genus with *A. flavus* and *A. parasiticus* strains leads to the formation of aflatoxins [11]. The aflatoxins can be secreted as exotoxins or produced in fungi's spores and mycelium [12].

Mycotoxins are known to trigger a wide range of health problems concerning vital organs [13] [14]. They may have a swift effect, or their long term exposure at relatively low concentration results in chronic problems [15]. Several occurrences of jaundice have been reported from India, Kenya, and Malaysia [16] [17]. Amongst the aflatoxin, AFB₁, and AFB₂ are considered to be the most toxic and dangerous aflatoxins [10].

Aflatoxins interfere with enzymes and substrates, affecting the critical processes leading to protein synthesis [18]. According to International Agency for Research on Cancer (IARC) [19] and Bennet J. W [11], both aflatoxin B₁ and B₂ are carcinogenic and have been placed in Group 1 of cancer-producing substances [11] [19]. Uptake of sufficient quantities can be highly carcinogenic and acutely toxic or fatal [20] to both humans and livestock. The worst affected organ by aflatoxins is the liver [21]. The earlier studies report that continued consumption of aflatoxin-contaminated foodstuff can lead to liver cancer [21]. It has also been reported that the aflatoxin-contaminated feeds enable metabolic biotransformation of AFB₁ in aflatoxin M₁ [22]. The aflatoxin M₁ and M₂ are possibly carcinogenic for humans and have been included in IARC Group 2A of cancer-producing substances [9] [19].

Fish happens to be one of the leading sources of protein in Zambia. Moreover, fish trade also affords a significant source of earnings to many Zambians. Further, a good number of people in Zambia rely on fisheries for their livelihood. Traditional methods are quite famous for fish processing, preservation, and storage in Zambia. Interestingly, a large volume of fish uses traditional methods for processing and preservation.

Some of the most notable traditional methods used in fish processing and preservation include smoke-drying and fermenting. However, smoke-drying is probably the most popular method, and therefore a large volume of the fish is consumed in smoke-dried form. In the rural areas where there are no refrigeration facilities, the smoke-dried fish happens to be the best option as the source of protein. The preference for the smoke-drying method lies in the fact that it is

low-cost and easy to carry out.

There are some earlier studies reported on the concentration of aflatoxins in dried fish [23] [24] [25]. The impact of aflatoxin-contaminated fish feed has also been studied. According to this study, the fish fed with contaminated feeds can acquire mycotoxins [26].

The earlier study in this area is scarce and insufficient. There is a dearth of information regarding aflatoxin contamination into most of the commonly consumed fish species in Zambia. Moreover, aflatoxins are an acute health hazard. Further, smoke-dried fish is consumed by a significant population in Zambia. Therefore, it would be exciting and significant to quantify aflatoxin concentration in this foodstuff. As such, this study aimed to appraise the prevalence of aflatoxin contamination in commonly consumed fish in Zambia and to highlight the risk posed to the people. This study also considered fresh fish samples to explore aflatoxin contamination, if any, through unknown aflatoxin-contaminated feeds.

2. Materials and Methods

2.1. Sample Collection

The samples for five types of smoke-dried fish were collected from three provinces of Zambia. The markets selected for sample collection include Chibombo-fish Market (CMO) in Central Province, Chisamba-fish Market (CSM) in Central Province, and Lusaka-Soweto Market (LSK) in Lusaka province of Zambia. Five species of dried fish were sampled randomly at each market. The species considered include bream (*Pharyngo chromis acuticeps*), Buka (*Lates stappersii*), Mintesa (*M. macrolepidotus*), Catfish (*Clarias gariepinus*), and Makobo (*Serranochromis*). The sample collections for fresh fish were carried out from superstores. The sample collection for this category was carried out from Copperbelt province (Mukuba Mall), Central Province (Kabwe Mall), and Lusaka Province (Manda Hill Mall).

2.2. Sample Preparation

The fish tissue samples were cut using a clean knife and placed into a blender. The sample was then ground at high speed. It was subsequently kept at -25°C . The sample was then brought to room temperature and extracted using the VICAM method as stipulated below.

2.3. Equipment Calibration

The analysis of fish samples used VICAM Series-4EX Fluorometer for the determination of the aflatoxins concentrations. The concentrations were obtained in the ppb unit. The standard calibration mode for Afla 50 for peanut was followed to calibrate the fluorometer. The calibration check of the system was carried out, and the percent recovery of this method was calculated to be 103.8% $(5.4/5.2) \times 100\%$. After calibration, each sample was tested for total aflatoxin concentration.

2.4. Sample Extraction and Analysis

2.4.1. Smoked Dried Fish

A 50.0 g of each ground sample was weighed and subsequently transferred to a high-speed stainless steel blender. A 5.0 g of sodium chloride (NaCl) salt was weighed and dissolved in 100 mL of a methanol-water mixture (80:20, v/v). This solution was also transferred to the jar containing the ground fish sample. The blender was covered, and the sample mixture in the blender was further blended at high speed for one minute. Further, the cover was removed from the jar. The extract was poured into a fluted filter paper, which was fitted with a funnel in a clean vessel. A pipette measured 10 mL of the filtered extract, which was poured into a clean vessel. The extract was diluted with 40 mL of wash buffer containing 0.1% tween PBS. The diluted extract was then filtered using a 0.1 µm filter paper.

Further, 10 mL of the filtered dilute extract was allowed to pass entirely through an AflaTest immuno-affinity column (IAC) (VICAM, Watertown, MA) at a flow rate of 1 - 2 drops per second until air came through the column. Further, 10 mL of deionized water was passed through the column at a rate of about 2 drops per second. This step was repeated by passing 10 mL of deionized water through the column at a rate of 2 drops per second until air came through the column. All the liquids were removed from the column by applying downward pressure using a pump through the syringe. The aflatoxin was eluted from the affinity column by passing 1.0 mL of HPLC grade methanol through the column at a rate of 1 - 2 drops per second and collected all the sample eluate (1.0 mL) in glass cuvette. A 1.0 mL of Afla Test Developer was added to the cuvette and then vortexed to mix thoroughly. The cuvette was covered with aluminium foil after adding the developer to avoid exposure to the light. After 60 seconds, filtration was carried out using nylon filters into the new cuvette. The cuvette was placed in the calibrated fluorometer. The aflatoxin concentration was noted after 60 seconds.

2.4.2. Fresh Fish

A 70.0 g of each sample was weighed. The higher sample weight considered in this case was prompted due to relatively higher water content in the fresh fish samples. It was then dried at 105°C. After drying, the sample was weighed to be 37.7 g. A similar extraction and analytical procedure was followed in this case, as described earlier for smoked dried fish samples above.

3. Results

3.1. Concentrations of Aflatoxins in Smoked Dried Fish

The total aflatoxin concentration, as obtained in this study, lies in the range from 1.3 ppb to 2.84 ppb. The Buka (CSM) showed the lowest aflatoxin concentration of 1.3 ppb, and Bream (LSK) had the highest aflatoxin concentration of 3.84 ppb.

The concentrations obtained for all the fish samples are stipulated in **Table 1** below. The concentrations are expressed in part per billion (ppb).

Table 1. Concentration of aflatoxins in smoked dried fish.

Sample name	Sample Concentration (ppb)
Buka LSK	1.96
Buka CSM	1.30
Buka CMO	1.80
Bream LSK	3.84
Bream CSM	3.78
Bream CMO	3.83
Mintesa LSK	2.60
Mintesa CSM	2.60
Mintesa CMO	2.60
Catfish LSK	3.40
Catfish CSM	3.40
Catfish CMO	3.30
Makobo LSK	1.60
Makobo CSM	1.60
Makobo CMO	1.50

The results for smoke-dried fish samples have also been depicted in **Figure 1** below.

3.2. Concentrations of Aflatoxins in Fresh Fish

There has been no trace of aflatoxins observed in all the six samples of the fresh fish.

4. Discussion

There are many regulatory frameworks for aflatoxin concentrations. These guidelines are variable with geographical divisions, such as Europe, Africa, Asia, USA, Canada [27] [28]. Because of the broader consumption of dried fish in Zambia, this study focused on quantifying aflatoxin presence and the health hazard it might pose. Moreover, no comprehensive study on smoke-dried fish seems to have been carried out in Zambia. Therefore, it was relevant to carry out this study.

Results obtained from this study show a considerable occurrence of aflatoxins in the dried fish sold in Chisamba, Chibombo, and Soweto markets in Zambia. The aflatoxin levels were found to range from 1.3 ppb to 3.84 ppb. The bream contained the uppermost value of 3.84 ppb of aflatoxins. The concentration levels are a matter of concern. However, they do not exceed the WHO limit. Although the concentration levels are below the permissible limit, the continued exposure can have a severe effect on human health.

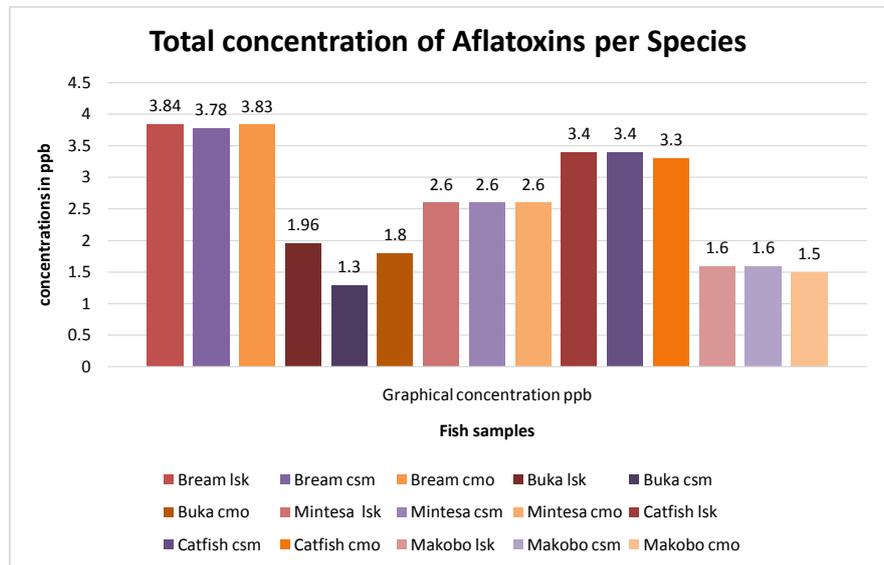


Figure 1. Total concentrations of aflatoxins in fish samples. **LSK** = Lusaka, **CSM** = Chisamba, **CMO** = Chibombo.

The results obtained in this study are not very far from the one produced by a previous study [23]. Another study [29] reported much lower aflatoxin concentrations from 0.030 ppb to 1.150 ppb. The relatively higher amount of aflatoxins in fish samples from Zambia may be attributed to the specific environmental conditions. A study [30] shows that AFs producing fungi grow drastically in moist conditions. Therefore, Zambia, having almost six months of the humid and moist rainy season, is exceptionally favorable for thriving fungi.

A relatively higher concentration in breams may be attributed to the fact that it is relatively expensive. Hence, it has a longer shelf life to favor fungi growth. Often, retailers display the smoked-dried fish samples in trays or heaps, and this favors fungal attack and production of toxins [23].

The purpose of studying fresh fish was to confirm if any trace of aflatoxins were present due to unknown feed factors. However, as per this study, Aflatoxins were not detected in fresh fish. Nevertheless, this study may not assert the absolute absence of aflatoxins conclusively because the method used involved heating at higher temperatures. The studies have established that aflatoxin concentrations drop drastically with temperatures [31] [32]. An alternative method merits to be developed before proclaiming fresh fish absolutely aflatoxin free.

5. Conclusions

The concentrations of the aflatoxins in all the fish samples in this study were relatively high but did not exceed WHO limits. However, the amount of aflatoxins found in this study can be a serious health hazard if consumed for an extended period.

The findings are not drastically variant to previous studies. The relatively higher concentration can be attributed to the prolonged wet and humid Zam-

bian rainy season. As AFs producing fungi thrive in moist conditions, a reliable monitoring system to ascertain the contamination level seems to be a necessity to protect people from the harmful effects of aflatoxins. Fresh fish did not show any concentration of aflatoxins.

The findings of this study indicate that prolonged consumption of smoked dried fish may have a severe health hazard to humans in this country. Further study to include many other fish types merits immediate attention. This study may attract further study to explore better methods of storage and display to reduce and control aflatoxin contamination. There is also a need to investigate the extent of health damage caused by aflatoxins in the Zambian perspective.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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