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Research Article

Mutual Occurrence and Dietary Exposure to Total Aflatoxin and Fumonisins in Bread: A Major Breakfast Bakery Product in Nigeria

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Abstract
Bread, which is a major breakfast food, has been documented to be susceptible to contamination by toxic fungi metabolites (mycotoxins) in various parts of the world. Total aflatoxins (AfT) and fumonisins (FB) are two important mycotoxins known for their ability to cause health damage to animals and humans when ingested through food over a long time. This study set out to determine the presence and level of these mycotoxins in bread produced and/or consumed within Lokoja, Kogi State, Nigeria. After extraction, ELISA was used to quantify the toxins in 30 samples of bread. The outcome showed 50% (15/30) contamination of bread samples by AfT and 93.3% (28/30) contamination by FB within the ranges 0.1-5.5 µg/kg and 10-220 µg/kg respectively. Three (3) of the bread samples were contaminated by AfT beyond the safe limit, while all the samples contaminated with FB were contaminated within the safe limit. The mutual occurrence of both toxins was recorded at 46.7%. EDI for AfT and FB were estimated to be 0.0048 µg/kg bw/day and 0.3379 µg/kg bw/day respectively while risk characterisation gave an estimated TDI% of 16.896% for FB. The results suggest that chronic toxic effects rather than acute toxicity could occur from long-term exposure to AfT and FB from bread. It is therefore advised that the raw materials used in bread manufacturing should be monitored and regulated for mycotoxins.

Key words: Bread, Fumonisins, Total Aflatoxin, Lokoja-Nigeria.

Introduction

The bread and biscuit bakery industry accounts for about 82% of total bakery products in Nigeria. This includes both the organised bakery sector including the large, medium and small-scale manufacturers and the unorganised bakery sector including small bakery units, cottage and house type manufacturers whose sales are mostly locally [1]. According to Nicole *et al*. [2] South Africa, Nigeria, Ethiopia, Sudan and Kenya are currently the largest and leading bread markets in Africa, and the demand is mostly driven by rising population, an expanded middle class with more money to expend, increase rural to urban relocation and an enlarged labour force. In Nigeria, bread is a staple made by baking dough of flour and water [3]. It has gained acceptance as a major breakfast food mainly by the town and city dwellers. Bread is presented in various forms, packaging, sizes, colour, texture, taste, shape, ingredient lists, and even cost, thus informing consumer's decision on which product to go for. Bread producers use flour (mostly white), sugar, salt, yeast, butter, Enzyme Development Corporations (EDC) baking enzymes, preservatives, milk, flavour, and water, most of which are not produced locally within the factory, but imported. Due to its moisture content and chemical composition, bread has a relatively short life-span. The nutritional composition of bread being mainly caloric (carbohydrate and fat) in addition to raw material factor, manufacturing and

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handling practices, and environmental factors predisposes it to fungi infestation, and resultant mycotoxin production, also since bread are mostly sliced they provide a large surface area which further enhances fungi growth.

Mycotoxin is the generic name for poisonous chemicals produced on crops and their products by fungi. They are found in almost all regions of the world. Of the over 500 mycotoxins currently known, only a few are currently regulated and monitored routinely [4,5]. They are a major contaminant of the world's foods, including 25% of the world's crops [6]. Mycotoxins are produced by fungi when presented with suitable temperature, relative humidity, and water activity. According to Legan [7], a relatively high water activity (a_w = 0.94-0.97) with a pH of approximately 6 possessed by bread makes it a good substrate for a wide range of fungi. The occurrence of moulds has been reported in bread [8,9], the occurrence of mycotoxin have also been reported [8, 10, 11, 12], also dietary exposure and risk characterisation of mycotoxins from bread has been reported [13, 12].

The health implications associated with the consumption of mycotoxin contaminated food are vast and life-threatening, ranging from acute toxicity to chronic toxicity. Among the over 500 known mycotoxins, AfT and FB rank within the first 5 in importance, they have both proven to be of concern to animal and human health, agriculture and trade. AfB1 the major component of AfT is among the International Agency for Research on Cancer [14] list of group 1 carcinogens, it also causes toxic hepatitis, haemorrhage, edema and immunosuppression [15] while FB are listed in group 2b, as a probable human carcinogen, they have also been reported to be hepatotoxic, nephrotoxic, embryotoxic and teratogenic in lab animals [16, 17]. *Aspergillus flavus*, *A. parasiticus* and *A. nomius* [18] which usually infect cereal crops including corn, wheat, peanuts, walnut, cotton and tree nuts [19] are well known producers of aflatoxins, while *Fusarium verticillioides* and *F. proliferatum*, which are well-established fumonisin producers, are dominantly cereal pathogens found worldwide and cause seedling diseases, and rot to stalk, root, kernel and ear [20,21]. Consumption of food which is inevitable, is the key path of human contact with mycotoxins [22].

In order to mitigate these risks, an initial step will be to bridge the knowledge gap by making incidence and occurrence data available in a manner that it is useful to decision makers at regional and global levels. This has not been made available from our region. Kogi State has several bread factories responsible for the production of bread, making it one of the biggest states in North-Central Nigeria with a high production of bread. This is influenced by the population, the high demand and consumption rate of bread. Hence it is important to appraise the level of contamination of bread by AfT and FB and estimate respective human exposures to them in order to adopt informed control measures toward curbing the mycotoxin associated menace. This research will therefore determine the incidence and levels of AfT and FB, their co-occurrence in bread and evaluate the exposure risk among Lokoja bread consuming populace.

Material and Methods

2.1. Materials and Reagents

Materials used in this work include: Romer series II mill, STAT FAX Elisa Reader MODEL: 303 PLUS, AgraQuant® Aflatoxin, AgraQuant® Fumonisin (Romer Labs, Getzersdorf Austria), a Chinese manufactured portable scale and a 120 kg graduated O'Divine weighing balance. The reagent used was methanol (HPLC grade).

2.2 Sampling and Data Collection

The study area was Lokoja, Kogi State, a town with an average population of about 195,261 based on the 2006 census. Lokoja is referred to as the Pittsburgh of Africa being the confluence point of the two major rivers running southwards to the Atlantic Ocean. It is located 7.8023° north of the equator and 6.7333° east of the Meridian. The town is situated in the tropical wet and dry savanna climate zone of Nigeria, and temperature remains hot all year round.

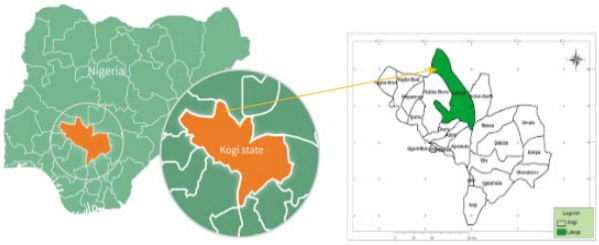

Figure 1. The map of Nigeria, showing Kogi and Lokoja
Source: https://www.synergos.org

Freshly baked bread samples were collected from various bakeries and shops into sample bags, sealed and the name, location, and ingredients of the bread taken. These bread samples represent 30 different bread manufacturers. The samples collected were tagged with the alphabets A-AD. The representative samples were air dried and then ground using a Romer series II mill so that 75 % will pass through a 20-mesh screen, and then thoroughly mixed.

Data Collection: A simple questionnaire was administered to 60 respondents to determine the frequency of bread consumption, and their consumption sizes. A portable scale was used to weigh bread samples. An O'Divine 120 kg graduated weighing scale was used to take the weights of several individuals within Lokoja.

2.3 Extraction of Total Aflatoxin and ELISA Quantification

A 70% methanol solution was prepared by making up 700 ml methanol to 1 litre. Twenty grammes (20 g) of each bread sample were weighed into a conical flask, into which 100 ml of 70% methanol was added to realize a 1:5 (w/v) proportion. The conical flask was capped and the mixture was shaken for 5 minutes. The extract was filtered through a Whatman No. 1 filter paper. The extract from above was used for AfT quantification. Conjugate solution (200 µl) was pipetted into dilution wells, 100 µl of each sample extract was added in to the dilution wells, mixed well and 100 µl from dilution wells was transferred into antibody coated wells and incubated at room temperature for 15 minutes, after incubation it was washed 5 times with distilled water and tapped to enable the wells dry up. Substrate solution (100 µl) was pipetted into the antibody coated wells and incubated for 5 minutes, after which 100 µl stop solution was pipetted into the antibody coated wells. The strips were read with a STAT FAX ELISA reader using 450 nm filter and 630 nm differential filters.

Limit of detection: 3 µg/kg

Range of quantitation: 4 – 40 µg/kg

2.4 Extraction of Fumonisin and ELISA Quantification

Twenty grammes (20 g) of each sample was weighed into a conical flask and 100 ml of 70/30 (v/v) methanol/water extraction solution was added, the flask was sealed and placed in the shaker for 3 minutes. The samples were allowed to settle and the top layer of extract was filtered through a Whatman No. 1 filter, the filtrate was collected in a glass vial. Conjugate solution (200 µl) was pipetted into dilution wells, where 100 µl of each sample extract was added into the dilution wells, mixed well and 100 µl from dilution wells was transferred into antibody coated wells and incubated at room temperature for 15 minutes, after incubation it was washed 5 times with distilled water, then tapped to dry the wells. Substrate solution (100 µl) was pipetted into the antibody coated wells and incubated for 5 minutes, after which 100 µl stop solution was pipetted into the antibody coated wells. The strips were read with a STAT FAX ELISA reader using 450 nm filter and 630 nm differential filter.

Limit of detection: 200 µg/kg

Range of quantitation: 250-5000 µg/kg

The ELISA reader generated a standard curve from where is extrapolates the level of mycotoxin in the samples and gave the final value based on the weight of sample. The curve was stored in the system (see Appendix 2)

2.5 Estimation of Dietary Exposure

The method used by Nugraha *et al* [23] and approved by JECFA was adopted in this study. This was achieved for AfT and FB based on their contamination level and the estimated consumption of bread in Lokoja.

Estimated daily intake (EDI) = *contamination level* x *consumption rate / Body weight (kg/ persons)*

Estimated daily intake = Estimate of the amount of toxin which can be ingested daily (ng/kg bw/day)

Contamination level = Mean toxin content in a certain foodstuff (ng/g)

Consumption rate = the amount of the foodstuffs ingested on daily basis (gram / day)

2.6 Risk characterization

The health risk characterization of each mycotoxin was performed by dividing the EDI previously calculated with the tolerable daily intake (TDI) (ng/kg bw/day) of the respective mycotoxins (when available) as indicated in the equation:

$$\%TDI = (EDI/TDI)*100$$

2.7 Statistical Analyses

Microsoft Excel package was employed. Mean and standard deviations, charts were used to present data.

Results and Discussions

Occurrence of Total Aflatoxins and Fumonisins in Bread

The presence of AfT was detected in 50% (15/30) of the samples tested (Table 1), however, only 10% of the bread samples were contaminated with AfT beyond the maximum residue level adopted by the Standards Organization of Nigeria (SON) as well as the European Union (EU); 4 µg/kg for AfT. The AfT content of bread sampled from Port-Harcourt, Southern Nigeria was reported within the range 0.80-1.73 µg/kg [11] which is in agreement with the range of 0.1-5.5 µg/kg found in this study. Much earlier than the report of Felagha *et al*. [11] from Southern Nigeria, Efuntoye in the year 2004 [8] analysed bread samples from Lagos, Ibadan and Ogbomosho Southern Nigeria for AfT, and found 11/150 samples representing 7.33% to be contaminated within the range 14 – 41 µg/kg far above the range of contamination found in this

study. Such a high range could be as a result of production practices and raw material issues back then, it is expected that the more stringent regulation and monitoring by the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC) would have led to improvement in manufacturing practices in a space of 12 and now 15 years respectively. In another study (AfT) was analysed in 237 breakfast cereal samples collected from central areas of Punjab, Pakistan, 41% incidence was recorded, of which 8% were found to be above the European Union (EU) maximum permissible limit for AfT, the authors found the highest mean level of AfT in semolina (4.55 µg/kg) [10]. Saladino *et al*. [12] reported AfT in 20% of 80 commercial bread in Valencia Spain including white, whole wheat and special bread loaves within the range 0.5 - 7.1 µg/kg. The incidence and range in their report is within the range of the report in this current report. Due to their stability to environmental conditions and through harsh processes, such as baking AfT are often detected in cereals cereal-based products [6]. The concentration of AfT found in this work may not be overtly alarming, however long-term chronic exposure to low levels of AfT in the diet is an important consideration for human health.

The presence of FB was detected in 28/30 samples which represents 93.3 % of the analysed samples (Table 1), none of the bread samples was contaminated with FB beyond the safe limit of 800 µg/kg. Umereweneza *et al*. [24] reported 67% and 44% contamination of maize flour by FB1 and FB2 respectively in Rwanda maize flour. Peanut flour had 11 % incidence of FB1 and cassava flour did not present FB1 [24]. Incidences of 60%, 51%, 42% and 4% have been reported in breakfast cereal, ethnic food samples (mean 202.9 µg/kg), breakfast cereal (mean 41.3 µg/kg) and cereal products [25, 26, 27, 28]. In another major bakery product being biscuit, FB level was found between the range of 36.5 to 75 µg/kg [22]. The level of FB found in this work may not be alarming because it is most likely not going to cause acute effects, but it is of concern also because of the chronic effects of long term FB exposure, which probably include carcinogenicity.

Table 1.

Incidence and Levels of Total Aflatoxin and Fumonisins in Bread from Lokoja, Nigeria

Sample Code	Total Aflatoxin (µg/kg)	Total Fumonisin (µg/kg)
Number of Samples Analysed	30	30
Number of positive samples	15	28
Percentage of positive samples	50%	93.3%
Range	0.1-5.5	10-220
Mean ± SD	1.71 ± 1.99	120 ± 60

Mutual Occurrence of Aflatoxin and Fumonisin

Fourteen (14) of 30 bread samples analysed contained both AfT and FB resulting to 46.7% joint occurrence. In all cases except in sample "W" FB levels were higher than AfT in all samples they jointly occurred (Figure 2). This would be the first report on the joint occurrence of AfT and FB in bread in Nigeria. The closely related works studied fungi contamination in bread; Unachukwu and Nwakanma [9] found *Rhizopus spp, Aspergillus spp, Mucor spp, Penicillium spp,* and *Fusarium spp* to be associated with the spoilage of bread. Efuntoye [8] reported *Rhizopus nigricans, Mucor mucedo, Aspergillus niger, Geotrichum albidum, Penicillium expansum, Aspergillus flavus, Rhizopus stolonifer, Alternaria sp* and *Aspergillus parasiticus* in brown bread. Without making conclusions, due to the limitation with sample size, it was observed that all samples that had wheat added (E, Y, and AD) had both AfT and FB content in them at varying concentrations. On the combined toxic effect of AfT and FB ingestion, Gelderblom *et al*. [29] and Carlson *et al*. [30] showed that AFB1 and FB1 interact synergistically in both cancer initiation and promotion, depending on intake conditions. In a study by Theumer *et al*. [31], subchronic doses of the AFB1 + FB1 mixture induced more pronounced apoptosis in the liver of male Wistar rats than either toxin alone. In addition, this combination provoked a significant increase in sphinganine (Sa) and sphingosine / sphinganine (Sa/So) ratio in the kidney and liver compared to FB1 alone, indicating that AFB1 enhanced FB1-induced impairments of sphingolipid metabolism. In another study by Theumer *et al*. [32] both toxins applied alone or in combination, affected the immune response of rats differently. Spleen

mononuclear cells (SMC) of rats fed with AFB1 + FB1 produced higher levels of IL-4 and lower levels of IL-10 compared to the SMC of animals fed with AFB1 alone. This suggests that subchronic doses of AFB1 and FB1 may produce an imbalance in T- helpers 1 and T-helpers 2 cellular subpopulations.

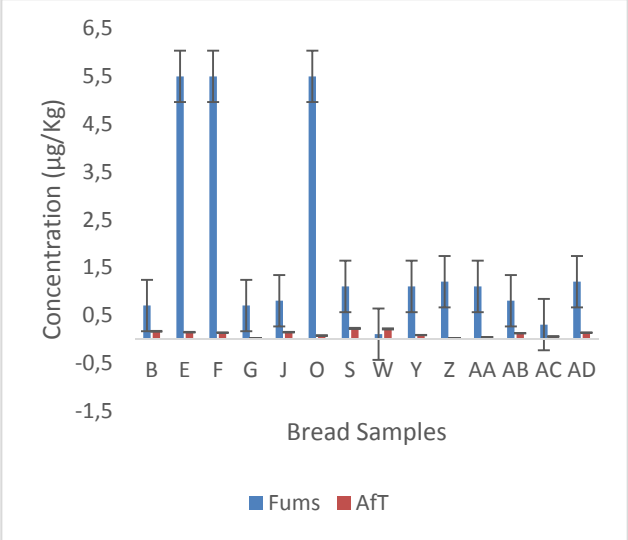

Figure 2. Co-Occurrence of Aflatoxins and Fumonisins in Bread Samples

3.3 Frequency of Bread Consumption in Lokoja, Nigeria

Considering the output from the respondents, 13%, 70%, 11%, 2% and2% of the bread consumers, consumed bread daily, weekly, monthly, quarterly and not-at-all respectively (Figure 3.0). Also on an average those who consume bread daily eat it once in a day, others eat it 4 times in a week, some eat it 7 times monthly while a few eat it 5 times within 3 months. We came in contact with individuals who do not eat bread at all, and this suggests that despite the availability of low sugar and whole wheat breads for dieters and diseased individuals that makes it possible for more people to consume bread, some people remain stereotyped non - bread consumers. We estimated an average consumption bread size at 176 ± 50 g / serving. Most breads are packaged in 100g, 200g, 500g, 800g, 900g among others.

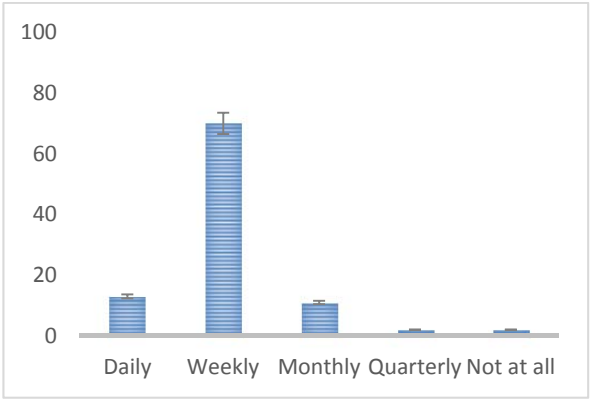

Figure 3. Frequency (%) of bread consumption in Lokoja, North Central Nigeria

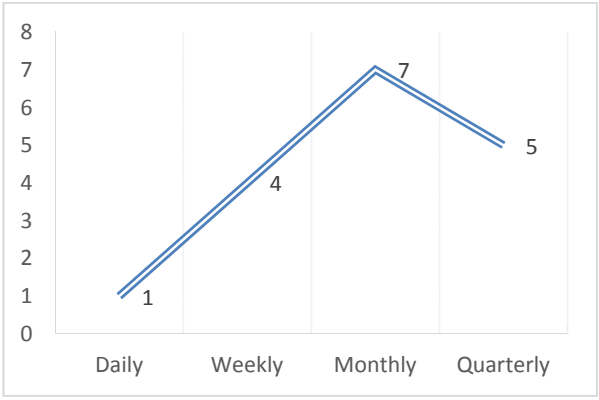

Figure 4. Average number of times people consume bread in Lokoja, North Central Nigeria

3.4 Estimation of Dietary Exposure and Risk characterization

The EDI of AfT and FB per bread serving which equals the EDI per day are estimated at 0.0048 µg/kg bw/day and 0.3379 µg/kg bw/day respectively. This was based on the estimations of average bread consumption size within Lokoja at 176± 56 g/day and the average weight of an adult in Lokoja at 62.5 ± 3.34 kg. to properly represent the weekly, monthly and quarterly intakes the EDI will then be multiplied by 4, 7 and 5 respectively (figure 4), rather than by 7, 30 and 90 which are the number of days that make up a week, month and quarter. On risk characterization using % TDI it was not needful to calculate values for AfB since no TDI was set for aflatoxins by JECFA being a carcinogenic. The “As Low As Reasonably Achievable (ALARA)” recommendation applies to such substances for which no completely safe level can be established. Therefore, the calculated EDI cannot be directly compared with tolerable level for AFs. Toxicological guidance value for FB was set by JECFA as a Provisional maximum

tolerable daily intake (PMTDI) of 2 µg/kg bw (2000 ng/kg bw) (JECFA, 2001 and 2011). Based on this value, the %TDI for FB in bread from Lokoja was estimated at 16.896%. Carballo *et al*. [33] reported EDI ranging from 0.32–103 ng/kg bw/day and a %TDI ranging from 0.016–5.15% in several published studies [22, 25, 26, 27, 28, 34].

The ingredient generally used in bread production within Lokoja Kogi State are flour (Honey well, Golden penny or Dangote), sugar (Honey well or Dangote), salt, yeast, preservatives, EDC 95, butter, vegetable oil, and water. It is possible for the flour, sugar or oil to be contaminated with any or both of the toxins under study before it was used in bread production. The outcome of this research showed 50% (15/30) contamination of bread samples by AfT and 93.3% (28/30) contamination by FB. Three (3) of the bread samples were contaminated by AfT above the safe limit, while all the samples contaminated with FB were contaminated within the safe limit. Co-occurrence of AfT and FB was also observed in 46.67 % of the samples analysed. The study suggests that rather than acute toxicity, chronic toxicity effects can result from long-term exposure to AfT and FB from bread.

Recommendations

We recommend a Hazard Analysis Critical Control Point (HACCP) along the bread value chain to identify the possible sources of AfT and FB into the product, it is also recommended that regulatory agencies should monitor mycotoxin contamination in the raw materials used in bread manufacturing. And finally, Bread should not be a sole source of breakfast food.

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