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Effects of fermentation process on levels of Aflatoxin B1 in Cereals

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Abstract

Aflatoxin contamination of cereals is of serious economic and health concern because of their toxigenic and carcinogenic effects. Biological detoxification and biodegradation of aflatoxin by microorganisms (bacteria and fungi) or their enzymes have been reported. In this study, a local fermentation strategy employed by farmers was mimicked using a native microflora, Saccharomyces cerevisiae, and Lactic Acid Bacteria (LAB) on different cereals contaminated with aflatoxin B1. In the experiment, cereal flours were fermented without any inoculum (chance fermentation), with Saccharomyces cerevisiae and with LAB separately. The setups were incubated at room temperature for three days. The aflatoxin B1 levels were analysed quantitatively before and after fermentation using Enzyme Link Immunoassay (ELISA). The results of the effects of fermentation types on the levels of AFB1 in the cereals revealed that the aflatoxin B1 levels in flour of millet obtained from the open market was reduced from 8.1±0.2ppb to 0.0±0.0ppb (100%) by chance and LAB fermentation while it was reduced from 8.1±0.2ppb to 0.2±0.01 by the action of Saccharomyces cerevisiae. Also, reduction in aflatoxin B1 levels was noticed in flour of stored millet after fermentation; native microflora and LAB reduced the aflatoxin B1 level from 7.5±0.0 to 0.0±0.0 while the level was reduced to 0.1±0.0 by Saccharomyces cerevisiae. Relatively low aflatoxin B1 reduction was observed in flour of stored wheat from 1.1±0.1ppb to 0.4±0.0 and 0.7±0.0 by native microflora and S. cerevisiae respectively, while total removal (from 1.1±0.1ppb to 0.0±0.0ppb) was noticed for LAB. Aflatoxin B1 bio-detoxification using the three types of fermentation showed that LAB had a 100% reduction in almost all the sampled stored market cereal flours with the exception of sorghum (from 7.9±0.1 to 0.6±0.1 ppb), rice (7.5±0.0 to 0.7±0.1 ppb) and sesame (1.2±0.0 to 0.1±0.1 ppb). These results showed that chance fermentation was as effective as fermentation with Saccharomyces cerevisiae but, relatively low compared to LAB fermentation only. Pronounced reduction in aflatoxin B1 levels in flours of cereals in this study after fermentation by LAB, suggests that it is a better bio-detoxifier and should be added during local fermentation by farmers.

Keywords: Aflatoxin B1, Cereals, Fermentation, LAB, Native microflora, *Saccharomyces cerevisiae*

INTRODUCTION

Mycotoxins have been linked to cereals as a key dietary exposure route, especially when they persist in subsequent products made from these grains (Makun *et al.,* 2010; Chala *et al.,* 2014). Important cereal crops that are widely grown in Africa include sorghum, millet, and wheat. However, throughout cultivation, harvest, storage, and processing, these crops, like other cereal crops, are vulnerable to fungal growth. These crops' colonization by toxic fungi may

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result in the formation of secondary metabolites, such as mycotoxins (Chala *et al.,* 2014; Taye *et al*., 2018).

Mycotoxins, including aflatoxin, have affected most crops grown worldwide; however, the extent of aflatoxin toxicity varies according to the commodities (Abbas *et al.,* 2010). Aflatoxin can infect crops during growth phases or even after harvesting (Kumar *et al*., 2016). Exposure to this toxin poses serious hazards to human health

For the detoxification of mycotoxins in foods, several methods have been developed (Shekhar *et al.,* 2025). In general, there are three ways to avoid the negative effects of mycotoxin contamination of food and feed: preventing contamination; decontaminating food and feed that has been contaminated; and preventing or inhibiting the absorption of mycotoxin content of eaten food into the digestive system. Preventative interventions taken before and after harvest haven't always had the desired impact (Bata and Lasztity, 1999).

Numerous papers have reviewed in-depth the physical and chemical methods of mycotoxin decontamination (Huwig *et al*., 2001; Diaz *et al*., 2004; Shekhar *et al.,* 2025). Mycotoxin can be largely eliminated through milling and dry cleaning of the grains. Fractionation caused by milling resulted in a higher level of mycotoxin in the bran and a lower level in the flour. Since the majority of mycotoxin are heat stable, heat treatment, which is frequently used in the food industry, does not significantly affect the mycotoxin level. There have been efforts made in a number of nations to establish an economically feasible method of converting mycotoxins into non-toxic compounds utilizing various chemicals, including alkali and oxidative agents. Although this process lowers the mycotoxin content, it also causes some nutrients to be lost, making it too extreme for grains used in food. Numerous physical adsorbents have been investigated and are now commercially prepared as feed additives for animals. But many of these adsorbents can only bind to a restricted subset of toxins, while exhibiting little to no affinity to other toxins (Huwig *et al.,* 2001). Although some of the current methods have been somewhat successful, most systems have significant drawbacks, ranging from low efficacy to nutrient losses and typically high prices.

Mycotoxins have been biologically decontaminated and biodegraded using microbes or enzymes (Juodeikiene *et al.,* 2012). It has been demonstrated that numerous bacterial and fungal species may enzymatically breakdown mycotoxins (Lyagin and Efremenko, 2019). As no hazardous chemicals were employed in this instance, there were no significant reductions in the nutritional value and flavour of the decontaminated food and feed. Fermentation is one of the simplest and least expensive ways to preserve food, and it also has nutritional and organoleptic advantages for fermented foods. The fermentation process is influenced by natural microbiota of the raw materials, microbes attached to the fermentation apparatus, or starter cultures added to the raw materials. Yeast and lactic acid bacteria (LAB), are naturally occurring microorganisms that are present in starter cultures used in the food and beverage industries (Jespersen, 2003; García-Díez and Saraiva, 2021). Animals have been fed with yeasts for over a century, and currently there are numerous commercial yeast products being created specifically for animal feed (Celyk *et al.,* 2003). As a result, lactic acid bacteria and yeast have immense potential as a tool for addressing the issue of fungi/mycotoxins in foods made from cereal and animal feed.

Aflatoxin decontamination during fermentation was reported in several studies (Govaris *et al*., 2002; Hayo, 2018; Mukandungutse *et al.,* 2019; Wacoo *et al*., 2019). Aflatoxin B1 and AFG1 have been reported to decrease by around 50% during the initial stage of fermentation. It was explained by the toxin's degradation by microbes. During the brewing of beer, higher

reduction of aflatoxin B1 and OTA were recorded (Chu *et al.,* 1975). The AFB1 was detoxified both during the LAB-induced milk fermentation and the dough fermentation process used to make bread. A study by Govaris *et al.* (2002) showed that aflatoxin M1(AFM1) levels reduced between 13 and 22 % when cow milk was fermented to produce yogurt and by sixteen (16%) and thirty-four percent (34%) during storage for yogurt of pH 4.6 and 4.0 respectively.

In rural areas, it has become normal practice to store goods in poor conditions from October to July/August in order to sell them during the period of scarcity to maximize profits has led to AF contamination problems (Makun *et al.*, 2012). Other compelling factors that worsen the AF burden in Africa are public ignorance of the existence of the toxins; and introduction of contaminated food into the food chain which has become inevitable due to shortage of food supply caused by drought, wars and other socioeconomic and political insecurity (Wagacha and Muthomi, 2008).

Food processing treatments such as soaking, germinating and fermenting can be used to improve the nutritional quality of these contaminated cereals (Manzoor *et al.,* 2024). These food processing technologies if optimized can contribute also to the alleviation of micronutrient deficiency through the breakdown of anti-nutrients, such as phytate and protease inhibitors thereby increasing the enzymatic activity of protease enzymes which may have significant effects on degradation of aflatoxin.

Thus, it is expected that fermentation may be one of the methods used to lessen the health concerns linked to exposure to aflatoxins found in locally produced fermented products Therefore, this study aimed to determine the effects of optimized fermentation processes on the reduction of aflatoxin B1 in a bid to protect the safety and livelihood of consumers.

MATERIAL AND METHODS

Study Area

The study was conducted in Yobe state using grains collected from grain markets in Potiskum, Yobe state.

Collection of samples

A total of 24 cereal samples (50 g of each cereal sample) consisting of maize (4), millet (4), sorghum (4), sesame (4), wheat (4) and rice (4) were obtained from local grain markets in Potiskum, Yobe state. Samples were collected from stores and open market stores; the samples were collected from the bottom, middle and top of each sack into clean polyethene bags. The samples were labeled appropriately and stored at a room temperature in Microbiology Laboratory of Yobe State University, Damaturu until further analyses.

Determination of Aflatoxin B1 level in the Cereal Samples by Enzyme Linked Immunosorbent Assay (ELISA)

Following the manufacturer's instructions, Beacon Aflatoxin ELISA kits were used to measure the amount of aflatoxin B1 in the cereal samples.

After the grain samples were ground into a fine powder, 50.0 g of each sample were then added to a conical flask along with 5.0 g of NaCl. The materials were fully blended for three minutes at high speed (250 rpm) with 100 mL of 80% methanol. The mixtures were filtered once they had settled, and 5 mL of the extracts were then diluted with 20 mL of distilled water and filtered through a glass fiber filter.

The samples and reagents from the ELISA kit were warmed to room temperature. Each well received approximately 50 L of the HRP Enzyme Conjugate, followed by the addition of 50 L of the calibrator and sample extracts into the relevant wells.

Each well was filled with fifty (50) L of the antibody solution, the plate was carefully shake to mix the content and was then incubated for ten (10) minutes at room temperature. After the incubation, the contents of the wells were decanted and flooded with lab-grade water and decanted. Five washes were performed by repeating the wash process four times. The plate was placed upside down on absorbent paper, and the water wash solution was tapped out as much as possible. The addition of 100 µL of colorless substrate to each well came next. After a light shake to evenly distribute the contents, the plate was incubated for 10 minutes at room temperature. Each well received 100 µL of stop solution, which was then carefully mixed by shaking the plate.

A microtiter plate reader operating at 450 nm was used to measure and record each well's absorbance (or optical density, or OD). The amount of aflatoxin in the sample is inversely proportional to the color intensity (OD). A graph curve that was plotted using the OD of the calibrator wells and the concentration of the calibrator and levels of aflatoxin in the samples was obtained from it.

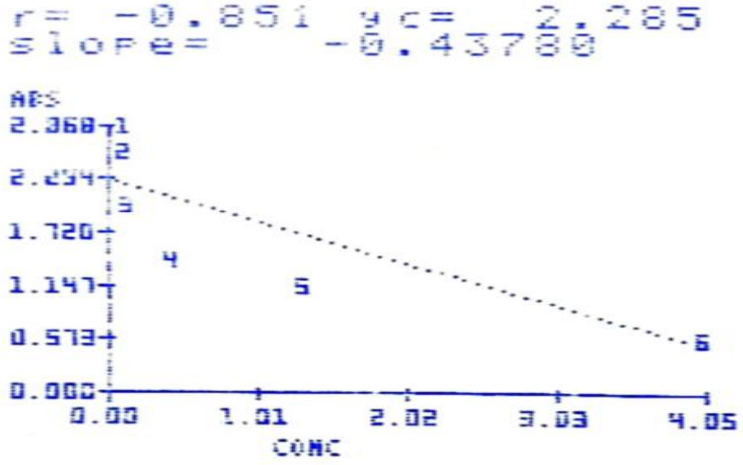

Fig. 3. Calibration curve of AFB1 with correlation coefficient

Determination of the Effect Fermentation on Aflatoxin B1 Level in cereals

Fermentation was carried out using grain powder to water ratio of 1:1.5 (w/v) and was done in three ways follows: After mixing of the cereal flour with sterile distilled water at the ratio of 1:1.5 (w/v), the set up was incubated at room temperature for 3 days for chance fermentation by native microflora. For fermentation through action of *Saccharomyces cerevisae*, the mixtures were inoculated with *Saccharomyces cerevisae* and incubated at room temperature for 3 days. While for fermentation through action of *Lactobacillus* species, the mixtures were inoculated with LAB and incubated at room temperature for 3 days. The levels of aflatoxin B1 in the fermented grains were determined using ELISA as described above in section 2.2.

Statistical analysis

The data generated in the course of this research were arranged in Microsoft Excel Package while Descriptive statistics was employed for data analysis. These include calculations of mean, standard error of mean.

Results

Detection of Aflatoxin B1 level in cereal

The incidence and level of aflatoxin B1 in stored and open market *cereals* is illustrated in Table 1. Aflatoxin B1 was detected in all four samples of millet with mean aflatoxin B1 level of 7.5±0.0 ppb. Aflatoxin B1 was detected in three out of the four samples of maize with mean aflatoxin B1 level of 5.0±0.1 ppb. Cereals with the least level of aflatoxin B1 were rice and sesame with mean aflatoxin B1 level of 1.3±0.0 ppb.

Table 1: Incidence and level of Aflatoxin B1 in stored and open market grains

Grain type n = 4	**Positive samples (%)**	***Mean aflatoxin level ± SE (ppb)**	
		Stored grains	Open market grains
Millet	4 (100.0)	7.5±0.0	8.1±0.2
Rice	3 (75.0)	1.3±0.0	7.5±0.0
Sorghum	4 (100.0)	4.5±0.0	7.9±0.1
Maize	3 (75.0)	5.0± 0.1	3.2±0.1
Sesame	2 (50.0)	1.3± 0.0	1.2±0.0
Wheat	2 (50.0)	1.6± 0.0	1.1±0.0

* NAFDAC acceptable limit is 10 ppb

Effect of Different Types of Fermentation on the Level AFB1 in Cereals

Table 2 shows the effect of fermentation type on the level of AFB1 in stored and open market. The level aflatoxin B1 in millet from open market was reduced from 8.1±0.2 ppb to 0.0±0.0 ppb, 0.0±0.0 ppb and 0.2±0.0 ppb by chance fermentation, fermentation by Lactic Acid Bacteria and fermentation by *S. cerevisiae.* The level of aflatoxin B1 in sesame from open market was reduced from 1.2±0.0 ppb to 0.8±0.1 ppb, 0.1±0.1 ppb and 0.5±0.1 ppb by chance fermentation, fermentation by Lactic Acid Bacteria and fermentation by *S. cerevisiae.* The level aflatoxin B1 in stored millet was reduced from 7.5±0.0 ppb to 0.0±0.0 ppb, 0.0±0.0 ppb and 0.1±0.0 ppb by chance fermentation, fermentation by Lactic Acid Bacteria and fermentation by *S. cerevisiae.*

Table 2: Effects of fermentation process in the level of AFB1 on stored and open market

Grain Type	Conc. of AFB1 before fermentation (ppb)	Concentration of AFB1 (ppb) after *fermentation by	
		(Chance)	(Lactic Acid Bacteria) (*S. cerevisiae*)
Open market grain			
Millet	8.1±0.2	0.0±0.0	0.0±0.0	0.2±0.1
Rice	7.5±0.0	0.7±0.1	0.7±0.1	1.2±0.0
Sorghum	7.9±0.1	1.4±0.0	0.6±0.1	1.0±0.0
Sesame	1.2±0.0	0.8±0.1	0.1±0.1	0.5±0.1
Maize	3.0±0.1	0.6±0.0	0.1±0.0	0.9±0.0
Stored grain				
Rice	1.3±0.0	0.3±0.1	0.1±0.0	1.0±0.1
Millet	7.5±0.0	0.0±0.0	0.0±0.0	0.1±0.0
Maize	5.0±0.1	0.2±0.1	0.1±0.1	3.0±0.1
Wheat	1.1±0.1	0.4±0.0	0.0±0.0	1.7±0.0
Sorghum	4.5±0.0	0.3±0.1	0.0±0.0	2.5±0.1

* Fermentation was carryout at room temperature for 3 days

DISCUSSION

Aflatoxin contamination of some cereals and grains has become somewhat inevitable as a result of intrinsic and extrinsic factors that favour fungus growth and aflatoxin production (Atanda *et al.,* 2011; Medina *et al.,* 2014; Smith *et al.,* 2016; Achaglinkame *et al.,* 2017).

Among the cereals contaminated, some had higher aflatoxin B1 levels compared to others. However, the aflatoxin levels in the contaminated grains were within the NAFDAC

acceptable limit of 10 ppb. Millet samples were the most contaminated with mean aflatoxin B1 levels of 7.5±0.0 ppb and 8.1±0.2 ppb for stored and open market millet respectively.

Furthermore, aflatoxin B1 levels recorded in wheat in this study (1.6± 0.0 ppb for stored wheat and 1.1±0.0 ppb for open market wheat) is low compared to 102.9 - 198.4 mg/kg aflatoxin B1 range in wheat reported by Makun *et al.* (2010) in Nigeria. This variation might be linked to different geographical locations, agricultural practices, and a variety of cereals and grains.

The mean aflatoxin B1 level in stored maize (5.0± 0.1 ppb) and open market maize (3.2±0.1 ppb) observed in this study is lower than the range of aflatoxin B1 in stored maize (270 ppb to 500 ppb) reported by Hassan *et al.* (2014) using ELISA. So also, a higher aflatoxin contamination range of 30.9 - 507.9 µg/kg (equivalent to 30.9 - 507.9 ppb) in maize from three agro-ecological zones of Nigeria was reported by Atehnkeng *et al*. (2008). However, lower aflatoxin levels in maize ranging between 0.55 - 0.46 µg/kg (equivalent to 0.55 - 0.46 ppb) were reported by Manjula *et al*. (2009). This difference might be due to differences in the maize varieties, physicochemical properties of maize samples, environmental conditions, and storage conditions.

The mean aflatoxin B1 levels in stored millet (7.5±0.0 ppb) and open market millet (8.1±0.2 ppb) in this study were higher than 0.08 - 1.40 µg/kg (equivalent to 0.08 - 1.40 ppb) aflatoxin levels in millet samples in Plateau State reported by Ezekiel *et al*. (2020). Differences in millet variety, storage conditions, agricultural practices, and environmental conditions might be linked to the difference observed in aflatoxin B1 levels in the studies. Aflatoxin B1 level of 1.32 µg/kg (equivalent to 1.32 ppb) in sorghum reported by Batagarawa *et al*. (2005) is lower than aflatoxin B1 levels in sorghum (4.5 – 7.9 ppb) observed in this study.

Growth of fungi and production of aflatoxin as well as other mycotoxins in cereals and grains are governed by many factors, however, the major determinants are climatic and storage conditions. This argues in favour of the variations observed in fungal diversity and levels of aflatoxin B1 contamination in grains in this study compared to other studies from different locations. Fungal and aflatoxin as well as other mycotoxins contamination in grains tend to be higher in locations characterized by hot and humid climates, improper storage conditions, and poor agricultural practices (Makun *et al*., 2010).

Reduction in aflatoxin B1 levels in cereals was observed in this study after chance fermentation, fermentation by Lactic Acid Bacteria, and fermentation by *S. cerevisiae.* LAB fermentation resulted in a higher reduction in aflatoxin B1 levels, suggesting that it is a better bio-detoxifier. The mechanism of aflatoxin removal by LAB has been reported to be through absorption of toxins to their cell wall components by non-covalent bonding (Adelekan and Nnamah, 2019) or opening up of lactone ring of aflatoxin B1 leading to complete detoxification (Mukandungutse *et al.,* 2019). So also, microorganisms can actively internalize and accumulate toxins (Adelekan and Nnamah, 2019). Similarly, aflatoxin B1 reduction by *S. cerevisiae* fermentation is said to be through the binding of toxic metabolites to its cell wall (Gonçalves *et al*., 2015; Chlebicz and Śliżewska, 2020).

Reduction in aflatoxin B1 levels after fermentation by Lactic Acid Bacteria observed in this study is in line with the findings of El-Nezami *et al.* (1998), Haskard *et al.* (2001), and Adelekan and Nnamah, (2019) who also reported a reduction in aflatoxin B1 levels after Lactic Acid Bacteria fermentation. Bio-detoxification of aflatoxin-contaminated sorghum and millet by Lactic Acid Bacteria was also reported by Ibitoye *et al*. (2020).

The reduction of aflatoxin contamination in cereals through yeast fermentation observed in this study is in line with the reports of Hayo (2018), Wacoo *et al.* (2019), and Mukandungutse *et al.* (2019). Furthermore, Mukandungutse *et al.* (2019) also observed a reduction in aflatoxin B1 concentration after spontaneous (chance) fermentation.

The performance and pronounce detoxification by *Lactobacillus* spp on levels of aflatoxin in this study at 100% reduction in millet flour from (8.1±0.2ppb to 0.0±0.0ppb) is similar to the findings of Oluwafemi *et al* (2012) reported that all maize grains infected with aflatoxin B1 were significantly reduced by LAB by more than 50% and that the potential of LAB species in reducing aflatoxin is mostly found in *Lactobacillus plantarum.*

The relative AFB1 reduction by *S. cerevisiae* on millet flour after fermentation from (8.1±0.2ppb to 0.2±0.01ppb) in this study may be due to the higher % of aflatoxigenic fungi (*A.flavus* 50%, *A. fumigatus* 50%) presence on the grains which contribute to the effects on the mitochondrial function of the yeast thereby slowing the oxygen utilization resulting in slow growth rate. This agreed with Juodeikiene *et al* (2012), who reported that *Fusarium* spp. contaminated with a high concentration of DON (3.95 $mgkg^{-1}$) negatively affected the fermentation of *Saccharomyces cerevisiae*. Fermentation by *S. cerevisiae* using grains (barley, corn, and wheat) contaminated with DON by Hanschmann and Krieg (2006) shows that DON was stable to the alcohol fermentation process and not metabolized by *S. cerevisiae.* The incomplete detoxification of the toxin by *S. cerevisiae* was attributed to the higher distribution of *Aspergillus flavus* in the cereals samples. Boeira *et al* (1999) reported that the presence of toxigenic fungi may inhibit mitochondrial function and causes slow oxygen utilization to the yeast resulting in slow growth rates. The Reduction in aflatoxin B1 levels in cereals through fermentation could be deployed by farmers as one of the strategies to reduce health risks associated with exposure to aflatoxins in local fermented products.

CONCLUSION

Almost 75% of the *cereals* in this study are contaminated with aflatoxin B1; some had higher aflatoxin B1 levels compared to others. However, the aflatoxin levels in the contaminated cereals were within the NAFDAC acceptable limit of 10 ppb. Biological detoxification and biodegradation of aflatoxin by lactic acid bacteria, native microflora, and *S. cerevisiae* were observed in this study and have proven to be promising. The LAB had higher potential as a bio-detoxifier of aflatoxin B1 in a bid to ensure the safety and livelihood of consumers.

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