Fermentation and Biocontrol of Mycotoxins in *Sorghum bicolor* L. Moench Sold in Selected Markets in Abeokuta Metropolis, Ogun State, Nigeria.

Adewunmi A. A*. and Chingoma G. I.
Department of Biological Sciences, McPherson University, Seriki-Sotayo, Ogun State, Nigeria.
* Corresponding author: annabella.adewunmi02@gmail.com

Abstract: *Sorghum bicolor* L. Moench (Guinea corn) is a cereal that is widely planted and consumed as staple in Nigeria. Mycotoxin contamination of this grain can present public health concerns to consumers especially children, where it can be fatal. This study investigated the use of fermentation as a biocontrol strategy to mitigate mycotoxins present in *Sorghum bicolor* grains. A total of 30 samples of sorghum were purchased from 3 selected markets in Abeokuta metropolis and pooled into 3 composites per market. Each pooled sample was divided into 2 parts. One part was allowed to ferment spontaneously for 96 hours, while the other part was left unfermented. These were screened for the presence of fungi using isolation method, and fungal metabolites using LC-MS/MS analytical method. The fungal species identified in non-fermented sorghum were *Aspergillus niger, Aspergillus. flavus, Aspergillus turbingensis*, and *Fusarium coffeatum*, while those in fermented sorghum were *Pichia kudriavzii* and *Candida parapsilosis.* Aflatoxin B1 and B2 were detected in the non-fermented grains with concentration of 41.6 µg/kg +/-0.02 (SE) and 5.77 µg/kg +/- 0.01 (SE) respectively, both above EU recommended limits. In the fermented grains, AFB1 and AFB2 were drastically reduced to 5.77 µg/kg and 1.54 µg/kg respectively. High amounts of fusaric acid (421 µg/kg) were also recorded in the fermented grains. The presence of fusaric acid in the fermented grains in high amounts could be of public health concern though moderately toxic to animals. The reduction in AFB1 and AFB2 content in the fermented samples indicates that fermentation can be employed as a biocontrol strategy for the mitigation of mycotoxins in cereal-based foods.
Key word: Fermentation, LC-MS/MS, mycotoxins, public health, *Soghum bicolor.*

INTRODUCTION

Sorghum bicolor L. Moench, commonly known as Guinea corn, is one of the principal staple cereal crops grown in sub-Sahara Africa. Sorghum is used as raw materials and ingredients for a variety of food products, which are consumed by all age groups including infants. Sorghum is also one of the most frequently contaminated grains with mycotoxigenic fungi (Osman *et al.*, 2017; Adewunmi *et al.*, 2021; Adewunmi and Fapohunda, 2022), leading to deterioration and reduction in grain quality, grain density, loss in seed mass, storage quality and seed germination (Waliyar, 2007). As a matter of fact, the conducive and favourable climatic conditions in Africa, comes with increased fungal proliferation, especially in grains and their food derivatives with attendant mycotoxin production. The occurrence of toxigenic fungi and mycotoxins in grains is a significant concern for food safety and public health. Therefore, the ingestion of such mycotoxin-contaminated grains by animals and humans can cause both acute and chronic mycotoxicosis, and cancers in man and animals (Vasanthi, 2018; Adewunmi and Fapohunda, 2022).

Contamination of mycotoxins can occur during any part of the complex food chain: pre-harvest, post-harvest, transportation, storage, and processing. This creates urgent demand for mycotoxin removal strategies to minimize hazards to consumers and food safety risks. The high risk associated with mycotoxins is due to their heat stability during the cooking process and the inability of the normal food procedures to remove them (Kabak, 2009). Decades of research have developed various approaches to detoxification of mycotoxins especially at the crop stage. Numerous chemical agents have been used for mycotoxin control such as organic acids, oxidizing agents, ammonia, hydrated oxides, and so on, which also inhibit mycotoxigenic fungal growth. According to Hwang *et al.* (2018) chemical and physical approaches can remove mycotoxins with various degree of success, but with attendant problems such as potential safety issues, loss of important

nutrients and high cost, which still hampers its application in the food industry. In recent years, biological detoxification approaches, particularly the use of microbial cells or enzymes have proven to be highly effective. Despite the extensive research on biological control of mycotoxins, the practical application in food and feeds matrices remain limited. In sub-Saharan Africa, fermented foods constitute a significant part of nutrition for the local populace. Fermentation is a natural process that has been used for thousands of years to preserve and enhance the nutritional value of food. Fermentation can transform grains into a wide range of fermented products such as bread, beer, iru, Pito, Ogi, brukutu, and so on. Fermentation of food confers desirable properties and improves food quality (Adebiyi *et al.*, 2019). Fermented foods have been labelled "functional foods" due to their substantiated health benefits and as such, their consumption has been increasingly encouraged (Adebiyi *et al.*, 2019; Makwana and Haiti, 2019). In this study, spontaneous fermentation process was used as a cheap, readily available and sustainable strategy for the control of mycotoxins present in sorghum grains in addition to the beneficial sensorial, nutritional and health benefits of the food.

MATERIALS AND METHODS

Sample collection: This study was limited to only major markets in Abeokuta metropolis, Ogun state, Nigeria namely: Lafenwa market, Kuto market and Omieda markets. *Sorghum bicolor* samples were purchased randomly from these three major markets. "One-cup" (100 g) each of sorghum grains were purchased from 10 traders randomly selected per market. Samples purchased were pooled per market to form 3 composites samples, sealed in sterile sample collection bags and taken for microbiological analysis.

Sample analysis: For the pre-analysis, 100 g of the pooled sorghum samples was weighed out and then divided into two equal parts. One part (50 g) of the sample was fermented with distilled water for 96 hours (4 days), while the other part was left unfermented. The fermented and non-fermented sorghum samples were crushed and ground into powder with a grinder.

Fungal isolation: One (1) gram each of the fermented and non-fermented sorghum samples were weighed into test tubes containing 9 ml of sterile distilled water. After thorough mixing with vortex mixer, 0.1 ml was transferred onto already poured and set malt extract agar (MEA) plates containing chloramphenicol (500 mg/L) and streptomycin (500 mg/L) and cultured using the spread plating technique. The plates were incubated at 25^{o}C for 5 days. After 5 days, fungal colonies from plates were counted, sub-cultured using the 3-point culture technique on fresh MEA plates for purification of the isolates. The plates were incubated at 25^{o}C for 7 days, for good sporulation to take place, and then used for morphological identification. Pure cultures of the isolates were sub-cultured onto cryovials containing MEA and incubated again for 7 days, after which the pure cultures were aseptically covered with sterile distilled water, and kept at 25^{o}C for molecular identification.

Mycotoxin analysis of samples:

Five (5) grams of both fermented and unfermented ground sorghum grains were sent to Patent Co. laboratory, Hungary, for mycotoxin analysis. Mycotoxins associated with the sorghum samples were analysed using the dilute and shoot LC-MS/MS technique as described by Malachova *et al.* (2014). Five grams (5 g) of ground sorghum samples were homogenized with 20 ml of extraction solvent (acetonitrile/water/acetic acid 79:20:1 v/v/v) in a 50 ml polypropylene tube. All samples were extracted for 90 minutes on a GFL 3017 rotary shaker and diluted with the same volume of the extraction solvent. The diluted extracts were directly injected into the LC-MS/MS instrument. Apparent recoveries of the analytes were determined by spiking 0.25 g of the five different samples. The spiked samples were stored overnight at ambient

temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and samples.

RESULTS

The fungal species isolated from non-fermented sorghum were mainly moulds with total counts ranging from 1.1 x 10^2 – 3.6 x10^2 sfu/ml (Table 1), while the fungal species isolated from fermented sorghum were predominantly yeasts with total counts ranging from 2.2 x 10^4 – 3.9 x 10^5 cfu/ml (Table 1). The fungal species were identified based on colony morphology and molecular characterization as *Aspergillus niger*, *A. flavus*, *A. turbingensis*, and *Fusarium coffeatum* (Plates 1, 2, 3, 4). The mycotoxin profile of fermented and non-fermented *Sorghum bicolor* samples using LC-MS/MS technique is shown in Figure 1. Another secondary metabolite, fusaric acid was detected at a mean concentration of 421 µg/kg +/-0.01 (SE) in the fermented grains than in the non-fermented where only 42.3 µg/kg +/-0.02 (SE) was detected.

Table 1: Total moulds /yeasts counts in non-fermented and fermented sorghum grains / market

Code no.	Location	Total moulds count in non-fermented sorghum (cfu/mL)	Total yeasts count in fermented sorghum (cfu/mL)
001	Kuto	2.6 x 10^2	3.2 x10^4
002	Omeida	1.1 x10^2	3.9 x10^6
003	Lafenwa	3.6 x 10^2	2.2 x10^6

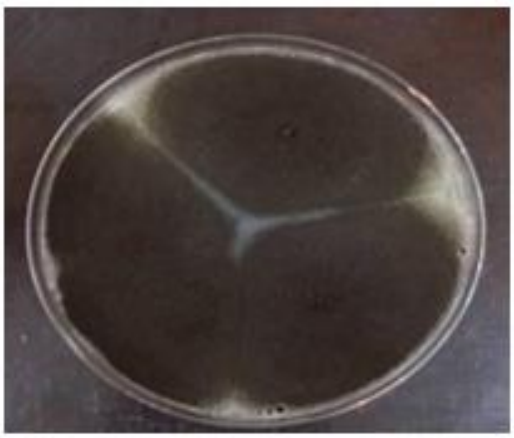

Plate 1: *Aspergillus niger* on MEA

Plate 2: *Aspergillus flavus* on MEA

Plate 3: *Fusarium coffeatum* on MEA

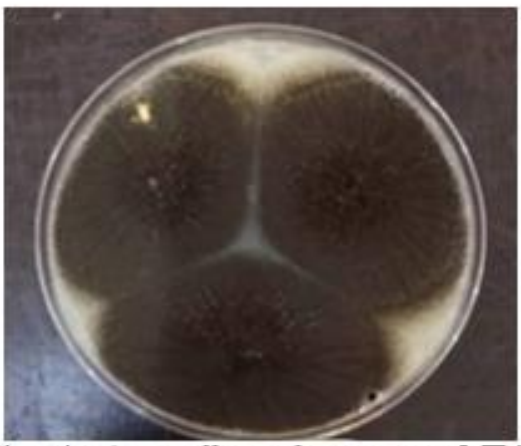

Plate 4 : *Aspergillus turbingensis* on MEA

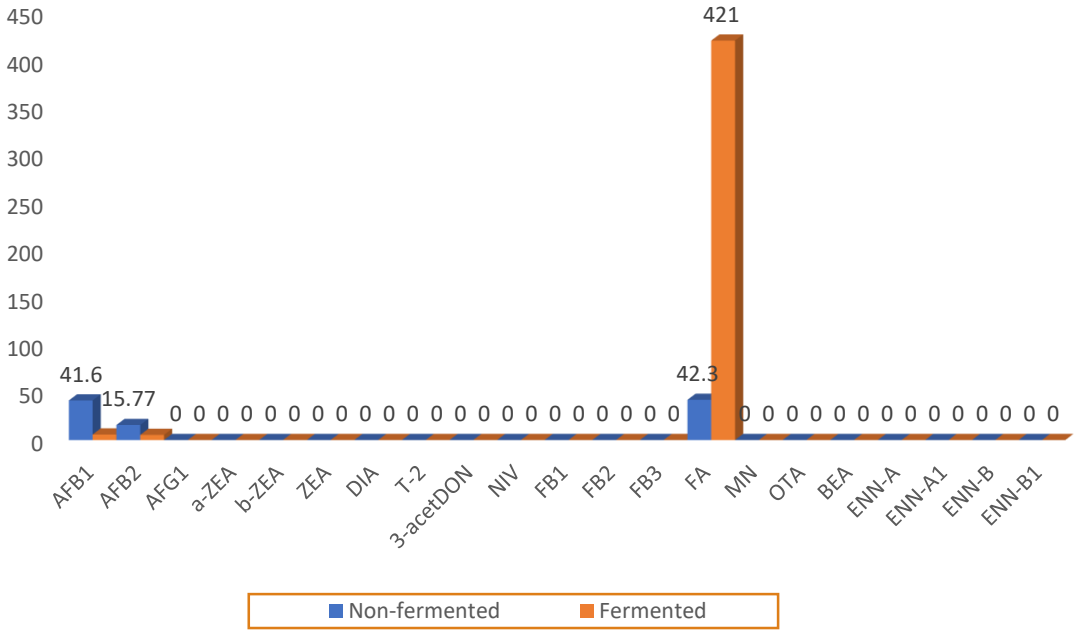

Figure 1: Mycotoxin profile of non-fermented and fermented sorghum grains

DISCUSSION

Aspergillus and *Fusarium* are filamentous fungi and are well known producers of both regulated and non-regulated mycotoxins (Osman *et al.*, 2017). The two species of yeasts were identified as *Pichia kudriavzii* and *Candida parapsilosis.*

Fermentation is a natural process that involves the conversion of organic compounds by microorganisms, such as lactic acid bacteria and yeasts under controlled conditions to other simpler compounds. This process has been utilized for centuries in the production of various food and beverage products (Taveira *et al.*, 2021). According to Aidoo *et al.* (2006), various yeast species have been reported as dominant species in the production of traditional fermented foods and beverages across the world. About 21 major genera with several species of functional yeasts have been reported from fermented foods that includes *Pichia, Saccharomyces, Rhodotorula, Candida*, and so on (Tamang and Fleet, 2009) and these yeasts are widely dispersed in nature. Therefore, the high total counts of yeasts in the fermented grains are expected.

A total of twenty-eight fungal metabolites were detected in both fermented and non-fermented sorghum samples at different concentrations. Two regulated mycotoxins, Aflatoxin B1 and B2 were detected in high concentrations in the non-fermented grains. The AFB1 recorded the mean concentration of 41.6 µg/kg +/-0.02 (SE), followed by Aflatoxin B2 with 5.77 µg/kg +/- 0.01 (SE), both above EU recommended limits of 2-4 µg/kg for cereals and Cereal products (EU, 2023). Other metabolites were present in concentrations below the detection levels. In the fermented grains, the concentrations of AFB1 and AFB2 were drastically reduced to 5.77 µg/kg +/-0.02 (SE) and 1.54 µg/kg +/- 0.01 (SE) respectively after four days of fermentation.

The findings in this study indicated that, there was a reduction in AFB1 and AFB2 contents in the sorghum samples after the four days fermentation process. Fermentation process can mitigate mycotoxin contamination through several mechanisms. Nathanail *et al.* (2016) observed that fermentative organisms can adsorb mycotoxins to their cell wall surface components, and may also enzymatically

transform mycotoxins into, generally, less toxic compounds. In other words, some microorganisms have the ability to bind or adsorb mycotoxin onto their own cell surfaces or into their cell walls. This binding reduces the bioavailability of mycotoxins and prevent them from being absorbed in the digestive system, thus reducing their harmful effect. Furthermore, certain microorganisms possess enzymes that can modify or degrade mycotoxins. For, example, some yeast strains produce enzymes like esterase and de-epoxidases that can transform or break down mycotoxins (Lyagin and Efremenko, 2019). Research has shown that fermentation with lactic acid bacteria (LAB) can be an effective strategy for mycotoxin degradation (Adebiyi *et al.,* 2019) because lactic acid bacteria can produce enzymes that can modify mycotoxin structures and reduce their toxicity. The AFB1 concentration decreased after 96 hours of fermentation, indicating possible mitigation of the toxic effect of the mycotoxin in the fermentation medium. Also, during fermentation, desirable microorganisms such as lactic acid bacteria, and yeasts can outcompete toxin-producing moulds, thereby reducing mycotoxin contamination in the fermented grains. In this study, moulds were isolated only from the non-fermented sorghum samples, while only yeasts were isolated from the fermented sorghum, indicating microbial competition and succession during the fermentation process.

Fementation often leads to the production of organic acids. These acids lower the pH of the fermentation environment, creating an acidic environment (Bao *et al.*, 2016). Many mycotoxin producing fungi are sensitive to low pH conditions, and the acidification can inhibit their growth and mycotoxin production (Daou *et al.*, 2021). In this study, high amount of fusaric acid was produced, with mean concentration of 42.3 µg/kg in non-fermented sample to 421 µg/kg in fermented sample, which lowered the pH of the fermentation environment, thereby inhibiting the growth of many moulds in the fermented sample. Fusaric acid has a low to moderate toxicity (Bacon *et al.*, 1996), but non-carcinogenic. Its presence in the fermented grains in high amounts could be of public health concerns because it might have synergistic effects with cooccurring mycotoxins (Porter *et al.*, 1990; Smith *et al.*, 1993). Microorganisms involved in fermentation can produce secondary metabolites that have antimicrobial properties. Fusaric acid has also been found to have antimicrobial properties. These antimicrobials can inhibit the growth and activity of mycotoxin-producing fungi, indirectly reducing mycotoxin levels (Porter *et al.*, 1995).

CONCLUSION

Fermentation of food confers desirable properties and improves food quality. In this study, this food processing technique, involving fermentation, has also proven to be a notable inexpensive mycotoxin decontamination strategy that could be explored not only to improve the constituents in food, but to reduce or eliminate mycotoxins. In the absence of sophisticated monitoring and prevention mechanisms in Africa, exploiting fermentation which is a traditional food processing method would also ensure food security and safety.

In addition, several techniques for mycotoxin control and management prove rather costly and/or impracticable in some instances, fermentation is a viable process for mycotoxin reduction in the African continent as well as other developing countries. This is due to its affordabilty, ease of the process as well as numerous other benefits the fermentation process confers on food. The AFB1 and AFB2 detected in the fermented grains were still above the EU limits even after four days of fermentation. Fermentation for longer periods of more than 4 days could reduce the mycotoxins content of the grains further to below EU limits.

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