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Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Isolation of toxigenic *Aspergillus flavus* and evaluation of aflatoxins in "Burukutu", sorghum fermented beverage sold in Akure, Nigeria

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ARTICLE INFO

Article history:
Received 29 Mar. 2019
Received in revised form
23 Jun. 2019
Accepted 02 Jul. 2019

Keywords:
Aflatoxins;
Aspergillus flavus;
Maize grains;
Bio-detoxification

ABSTRACT

The possible health threat accompanied with the ingestion of 'Burukutu', an alcoholic beverage made from fungal contaminated cereals grains calls for regular inspection. The study aimed at the isolation of toxigenic *Aspergillus flavus* and quantification of aflatoxins in'Burukutu' sold in Akure, Nigeria. The fungi associated with the samples were isolated and counted using standard microbiological methods. Aflatoxins were extracted from 64 samples using different solvents and analyzed with the aid of High Performance Thin-Layer Chromatography (HPTLC). The aflatoxins in the samples applied on pre-heated HPTLC plates were estimated under fluorescent UV light. Cooked fermented milled malted grains 'Burukutu' (CFMMG) had the highest fungal counts of 6.8×10^2 and 2.9×10^2 cfu/ml at 24 and 48 h of incubation respectively. The fungal isolates identified from the samples were: *Aspergillus flavus, Fusarium solani, Rhizopusstolonifer, A. fumigatus, A. niger, Penicillumitalicum, Saccharomyces cerevisae and Candida krusei*. All the analyzed samples showed varied quantity of aflatoxin concentrations. The overall quantification of aflatoxins G1, G2, B1 and B2 revealed significant reduction in end products 'Burukutu' when compared with the substrates from which it was made. Different general fungi associated with 'Burukutu' were identified. There was also a decline in the level of aflatoxins in 'Burukutu' which was a product of natural fermentation.

Citation: Olaniyi OO, Akinyele JB . **Isolation of toxigenic *Aspergillus flavus* and evaluation of aflatoxins in "Burukutu", sorghum fermented beverage sold in Akure, Nigeria.** J Food Safe & Hyg 2019; 5(1): 30-38

1. Introduction

'Burukutu' is a well beverage with a low alcoholic content that is characterized with vinegar-like flavor and a cloudy suspension; it is made largely from the grains of either *Sorghum bicolor* or *S. vulgare* (4,5). The grains of *Sorghum bicolor* or *S. vulgare* serve as a major food for the impoverished and less privileged individuals from the developing countries in which Nigeria is one and it is composed of energy and protein rich compounds (3). During production, 'gari' is often added to further enhance its aromatic properties. It is produced mainly at the cottage level by women who lack the scientific bases for its production. It can only be conserved for 8 days from the day of production, and its short shelf life might be linked to the presence of reduced lactic acid content, titratable acidity, alcoholic content, elevated content of vitamins and fermentable sugars (3).

Nigeria is a country blessed with good metrological conditions that favor the cultivation of cereals. However, enormous quantities of these cereals are wasted every year at the pre and post-harvesting stages due to certain favorable environmental conditions that favor the growth of mycotoxigenic fungal strains coupled with the lack of storage facilities (6,7). During the growth of these mycotoxin-producing fungi, varieties of toxic metabolites are released in varied amounts (6). The consequences of mycotoxin contamination are prominent in this part of the world where problem of food shortage and

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malnutrition already exist (8). The consequences are: food spoilage, destruction by mycotoxins regulatory agencies, rejection at the world markets and poor income generation. An attempt by farmers to integrate contaminated cereals into feed formulation might result in reduced growth rates, diseases in animal fed with such feeds and the products like meat and milk coming from such animals fed with fungal toxicant might contain harmful quantities (9,10). According to the data generated by Council for Agricultural Science and Technology (CAST) (11), world's food crop is affected by 25% to include many basic animal feed ingredients, cereals such as maize, rice and wheat.

Aflatoxins are the most prominent class of mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* and recently, its production had been confirmed in *A. nomius* (12). They are hepatotoxic in both human and animals. Out of the four main aflatoxins (B1, B2, G1 and G2) frequently encountered on cereals and their products, aflatoxin B1 was ranked as Class 1 human carcinogen because of its inherent mutagenic and carcinogenic properties (13,14). Coupled with the carcinogenic nature of aflatoxin B1, chronic health implicating risks have been associated to include immune suppression and deviation from normal blood and nerve functions (15).

Detoxification of aflatoxins has been investigated using various physical and chemical agents with varying degree of successes recorded (16,17). Attempted processes exploited to eliminate aflatoxins in contaminated foods and feed ingredients comprise of heat, gamma radiation, nixtamalization, ammoniation, sodium bisulfite and calcium hydroxide treatments (17,18). However, there were records of shortcomings attached to the use of these methods, and these include high cost, losses of nutritional value and undesirable health effects on consumers. Biological method is therefore considered to be the most attractive and safe for the elimination of aflatoxins in agricultural produces (17,19). In recent studies, the incorporation of chemical compounds (binders) to contaminated feeds as an aflatoxin detoxifying-agent has been reported to alter the hematological and histological status of the animals fed with them (18). With the afore-mentioned limitations, elimination of aflatoxins therefore remains unsolved problem that demands a search of novel detoxification methods applicable to feed and even to foods.

Different biological methods have been applied to prevent or suppress the growth of aflatoxin-producing fungi and to detoxify or bio-degrade aflatoxins (6,7). The several studies have demonstrated detoxification of aflatoxins in cereals and legumes by probiotic bacteria featured in food fermentation (17,20). According to Marrez et al. (18), different species of genus *Lactobacillus* detoxified aflatoxin B1 in milk, while Zinedine et al. (21) reported an appreciable reduction in aflatoxin B1 in Moroccan sourdough bread prepared with different strains of LAB. In a related study by Rayes (22), probiotic LAB removed aflatoxin B1 in artificially contaminated milk. Fermentation is a natural and safe biological control technique to safeguard foods and feeds against pre and post-harvest aflatoxin contamination (23). Previous studies major on the characterization of toxigenic *A. flavus* and quantification of aflatoxins from food and feed samples with no depth studies on this subject matter regarding '¸urukutu', an alcoholic beverage made from cereal grains. This study isolates toxigenic *A. flavus* and evaluates aflatoxins levels in 'Burukutu', sorghum fermented non-alcoholic beverage sold in Akure Metropolis.

2. Materials and methods

2.1. Sample collection

Samples of already prepared 'Burukutu', fermented milled malted grain, malted grain and sorghum grains were collected for four days at different selling points in Akure, Ondo State, Nigeria. Sixty-four samples were collected in sterile sealed containers and transported to the microbiology laboratory in ice parked for the isolation and enumeration of fungal isolates. The samples collected were divided into two portions in the laboratory and a portion was properly labeled for aflatoxins quantification.

2.2. Total heterotrophic fungal enumeration and identification

Tenfold serially diluted samples were surface plated in potato dextrose agar using appropriate dilution factors and incubated at room temperature 30^{o}C for 72 h. The resultant fungal colonies were added up and expressed as colony forming unit per millimeters ('Burukutu' and fermented milled malted grain) and colony forming unit per gram (malted grain and sorghum grains) as the case may be. Pure fungal isolates obtained after sub-culturing on the same agar medium were identified according to taxonomic criteria described by Barnett and Hunter (24). The identification of mold was on the bases of colonial features such as shape, colour and texture of colony,

and the presence of pigments) together with microscopic structure, while yeast isolates were identified by fermentative characterization and microscopic examination (24).

2.3. Aflatoxigenicity test on isolated *Aspergillus flavus*

Oat meal agar was compounded by dissolving 30 g and 6.25 g of oatmeal and agar respectively in 500 ml distilled water and sterilized in an autoclave at 121°C for 15 min. The sterilized agar medium was allowed to set and a pure culture of *A. flavus* was aseptically inoculated and incubated at 30°C in the dark cabinet for 7 days (25).

2.4. Preparation of fungal extract

Fungal extract was prepared by transferring plugs of *A. flavus* mycelia with the help of 10.0 mm cork borer in to Eppendorf tubes. The tubes containing the samples were kept in the freezer after which they broken and macerated with sterile spatula (26).

2.5. Extraction of aflatoxins

The extraction of aflatoxins was performed by the modified methods of Dinçkaya et al. (27). The macerated plugs was suspended in a solution and further broken into pieces in a mixer (Omni, Model 17106) for 30 min. The solution meant for extraction processwas prepared by mixing of 1.0 ml Dichloromethane with 1.0 ml of 1% acetic acid. The samples were later transferred into an ultrasonic water bath for 5 min after which they were subjected to centrifugation at 8,000 rpm for 10 min in a centrifuge (Labofuge A, Heraeus Christ, West Germany) to obtain the supernatant. The suspended particles and cell fragments were eliminated and the cleared supernatant was flushed with nitrogen gas in an evaporator at an ambient room temperature until it was dried. The sample was further dried by adding 0.5 ml methanol and re-flushed with nitrogen gas in an evaporator. It was reconstituted in 0.5 ml methanol and placed in an ultrasonic water bath for 5 min and re-centrifuged. Pipetted pooled fraction of the samples were aseptically collected in vials and kept in the freezer for High Performance Thin-Layer Chromatography (HPTLC) analysis.

2.6. Aflatoxins analysis through HPTLC

A computerized HPTLC machine was used to analyze for aflatoxins production in the samples. A calibration curve for each plate was prepared within the HPTLC machine. The mobile phase was prepared by mixing formic acid, Ethyl acetate and Toluene in ratio 1:30:60. The samples were applied on pre-heated HPTLC plates (at 70°C for 30 min) and the intensity of the aflatoxins was estimated under fluorescent UV light (28).

3. Results

3.1. Fungal counts

Figure 1 reveals the total aerobic fungal counts at 24 to 96 h of incubation. Cooked fermented milled malted grains 'burukutu' (CFMMG) had the highest fungal count of 6.8×10^2 and 2.9×10^2 cfu/ml at 24 and 48 h of incubation respectively while sorghum grains (SG) had least at 24 h and malted grains (MG) at 48 h. At 72 h of incubation, MG had the highest fungal population, followed by CFMMG and the lowest counts occurred in fermented milled malted grains (FMMG). The highest fungal load was obtained in FMMG and the least value occurred SG at 96 h of incubation.

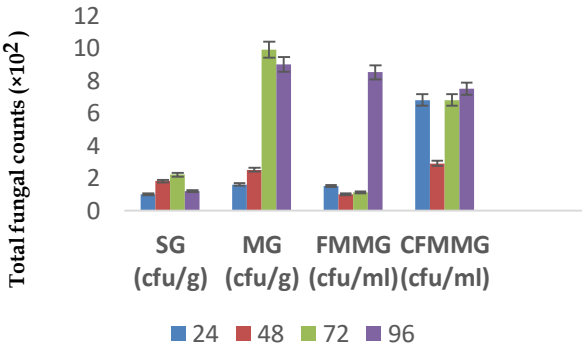

Figure 1. Total aerobic fungal counts from different stages of 'Burukutu' production
SG= Sorghum grains
MG= Malted grains
FMMG= Fermented milled malted grains
CFMMG= Cooked fermented milled malted grains (Burukutu)

3.2. Fungal identification and percentage occurrence

Table 1 presents the colonial features and microscopic observations of fungal isolates, while Table 2 reveals the fermentative pattern and morphological features of yeast isolates. The percentage occurrence of each of the isolates is presented in Figure 2. The fungal isolates identified were *Fusarium solani, Rhizopusstolonifer, Aspergillus flavus, A. fumigatus, A. niger, Penicillum italicum,*

Table1. Morphological and microscopic characteristics of mold isolates

Isolate code	Colony color	Microscopic observation	Suspected mold
1B2 and 2B4	Greenish Yellow	Greenish yellow mycelia growth and fully extended from the growth medium, upright conidiophores, terminating in a globose or swelling, swelling bearing phialides at the apex and radiating from the entire surface. Conidia (phialospores) is coloured in mass in basipetal chains.	*A. flavus*
3B4 and 3FB2	Brown mycelia	Conidiospores upright, simple, terminating in elevates, swelling, bearing phalides at the apex or radiating from the entire surface, L-celled globe often variously coloured in mathes in dry basipetal chains.	*A. fumigatus*
2MG4	Dark-grey mycelia	Spherical columella which borne sporangia on sporangiosphores.	*Rhizopus stolonifera*
1K2 and 4MG2	White mycelia with areas of whitish yellow	Colonies matured within 3 days. Fast growing rate with aerial mycelium. They appeared as sickle shaped.	*Fusariumsolani*
3E2	Blue mould growth	Septate mycelium bearing Single conidiophores which are branched near the apex ending in phialides that carries the conidia	*Penicilliumi talicum*
2B6	Black mycelia	Simple, upright conidiophores terminating in ovoid swelling	*A. niger*

Saccharomyces cerevisae and Candida krusei. S. cerevisae had the highest percentage occurrence followed by A. flavus and *C. krusei* with 70%, 30% and 20% respectively, while *A. fumigatus*, *A. niger, R. stolonifer*, *P. italicum* and *F. solani* had the same value of 10%.

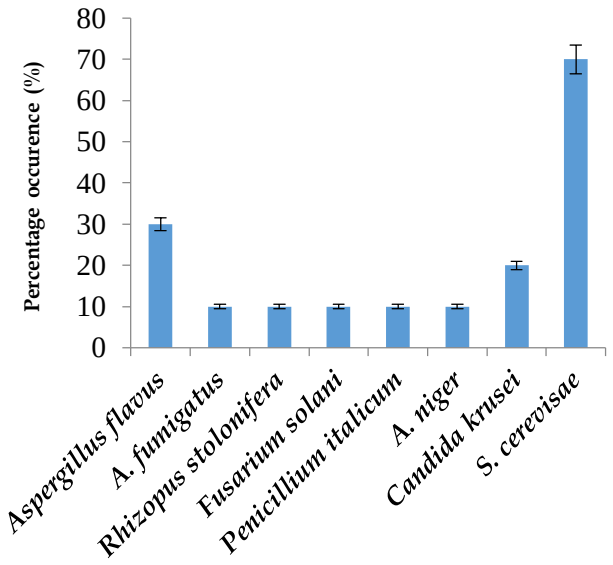

Figure 2. Percentage occurrence of fungal isolates (%)

Table 2. Microscopic features and fermentative pattern of yeast isolates

Isolated codes	Microscopic features	Sugar fermentation						Suspected Yeast
		GLU	TRH	FRU	XYL	LAC	SUC	
FB4	Narrow based budding spherical to ovoid blastoconidia	AG	NR	NR	NR	NR	NR	*Candida krusei*
K2	Large glabrous to ellipsoidal budding yeast-like cells or blastoconidia	AG	AG	AG	NR	AG	AG	*Saccharomyces cerevisae*
2K4	Large glabrous to ellipsoidal budding yeast-like cells or blastoconidia	AG	AG	AG	NR	AG	AG	*S. cerevisae*

AG-Acid and Gas production, NR-No Reaction
GLU-Glucose, TRH-Trehose, FRU-Fructose, XYL-Xylose, LAC-Lactose, SUC-Sucrose

3.1. Quantification of aflatoxins

Figure 3 shows the quantities of aflatoxins in ' Burukutu' at different stages of production (from the samples collected from 27th to 31st March, 2016). Out of all the classes of aflatoxins evaluated from the samples collected on 27th March, 2016, aflatoxin G1 had the least values of 0.026, 0.021 and 0.013 µg/kg for SG, MG and CFMMG, while B2 had the least value of 0.002 µg/kg in FMMG. There was a notable decline in all the classes of aflatoxins from SG to FMMG in all the samples collected on 27th March, 2016. Aflatoxins G1, G2, B1 and B2 decreased from 0.026 µg/kg in SG to 0.014 µg/kg in FMMG, 0.028 µg/kg to 0.016 µg/kg, 0.031 µg/kg to 0.018 µg/kg and 0.035 µg/kg to 0.002 µg/kg respectively. All the samples collected on 31st March, 2019 had the least values of aflatoxins G1, G2, B1 and B2 when compared with the quantities of these aflatoxins in samples collected on 27th, 28th March, 2016. The overall quantification of aflatoxins G1, G2, B1 and B2 revealed notable decrease in end product 'Burukutu' when compared with the substrate (sorghum grains) from where the beverage was made.

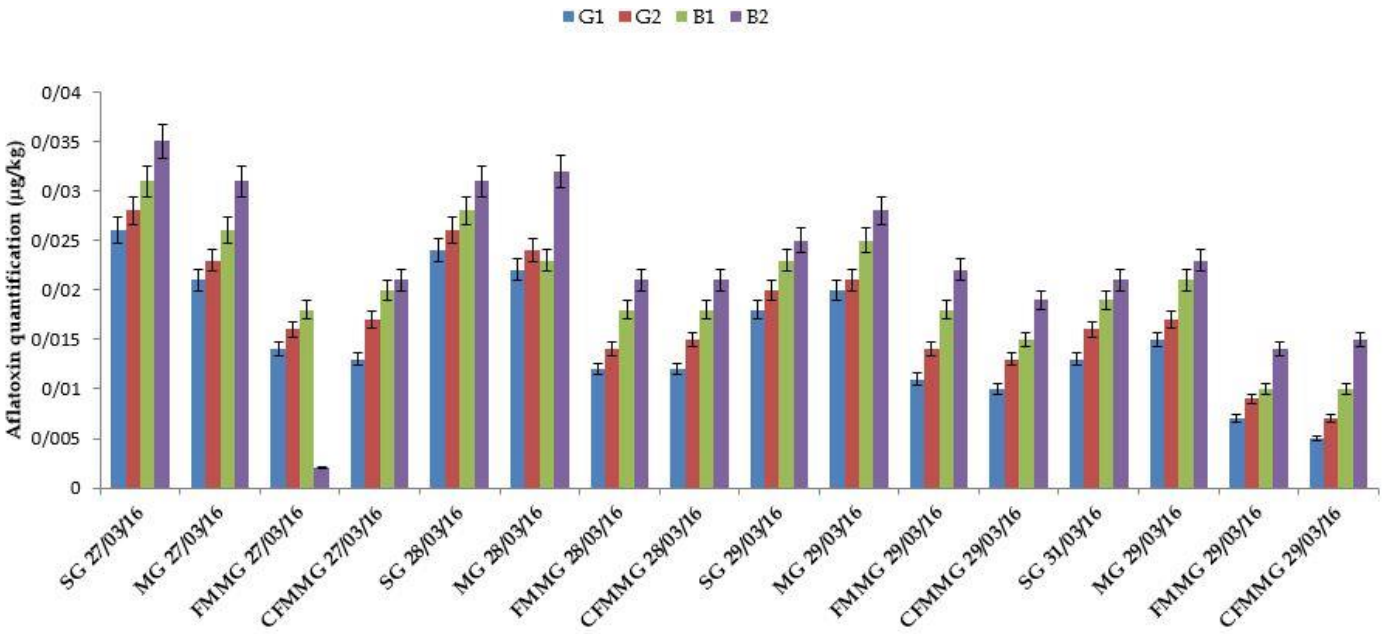

Figure 3. Quantification of aflatoxins in 'Burukutu' at different stages of production
SG= Sorghum grains
MG= Malted grains
FMMG= Fermented milled malted grains
CFMMG= Cooked fermented milled malted grains ('Burukutu')

4. Discussion

There were variations in the aerobic fungal population at different stages of 'Burukutu' production. In a study conducted by Ijabadeniyi (2007) (28), varied fungal populations were obtained during the fermentation of different maize varieties for 'ogi' production. Sulma et al. (2010) (29) reported different fungal loads from the Sudanese 'kisra', a traditional fermented sorghum based food. In a similar work by Abdel et al. (2017) (30), varied fungal populations were encountered during the fermentation of three sorghum varieties indigenous to Sudan for kisra bread. The present study aggress with the findings of Oriola et al. (2017) (31) who also reported varied fungal population from 'Otika', a Nigerian indigenous alcoholic beverage produced from sorghum. The sequential increase in the total fungal counts from the sorghum grains to malted grains might be connected with the contaminants sourced from raw materials from the farm, packaging utensils, personnel involved in the production processes and the production materials such as water sample, malting mat and so on. Higher fungal counts recorded at malting stage in comparison with the sorghum grains might be considered as the subject of more water activity. Increased moisture contents in food substrates area vital factor that facilitates the proliferation of microorganisms. Malted grains with higher moisture might be considered to possess nutritional qualities predisposing it to effective fungal attack. The reduced fungal counts at mid-fermentation stage were envisaged since certain organic acids and inhibitory substances are produced at this stage. Varied organic acids produced during fermentation by consortium of lactic acid bacteria had been reported byEfiuvwevwere and Akoma (1995) (32) and Akoma et al. (2006) (33), and this usually brings about the reduction in pH values. The resultant effect of low pH values could have facilitated varied fungal growth at different stages of production (34). The higher fungal loads obtained from cooked fermented milled malted grains 'Burukutu' might be due to the addition of spices with a view to improve its taste and flavor, and this could be a high entry point for different fungal species (35,36). In addition, high degree of fungal contamination could have occurred during packaging by unhygienic individuals who do not put in place necessary precautions and such avoidable contamination could be pronounced (35).

The identities of fungi associated with the production of 'Burukutu' revealed the presence of frequently encountered mold and yeast isolates in fermented cereals. There was a close agreement between this study and that of Oriola et al. (2017) (31) who also isolated mold and yeast at different stages of'Otika' production. According to Oriola et al. (2017) (31), *A. flavus*, *A. fumigatus, Penicillium italicum* and

S. cerevisaewere the dominant fungal isolates encountered. In a separate study by Efiuvwevwere and Akoma (1995) (32) and Akinleye et al. (2014) (37) arrays of fungal isolates were identified from traditional fermented sorghum flour of three local cultivars and 'Ogi' respectively. Different fungal isolates were identified from 'Oshikundu', a cereal based fermented beverage from Namibia (38). The identities of the associated fungi showed that the samples were exposed to different genera of fungi leading to their contamination. Fungi are known for their ability to utilize organic acids, and this might account their presence at different stages of production. The presence of fungus such as *A. flavus is* of great concern since it is known for aflatoxins secretion, a carcinogenic compound (39). The health of consumers might be impaired if higher dose of fuminosin, a toxin produced by A. fumigatus is consumed in food or feeds. The presence of *S. cerevisae* in samples evaluated could be connected to the low oxygen tension during the course of fermentation. The proliferation of yeasts under low oxygen tension during fermentation had been reported by Umaru et al. (2014) (40). Achi (2005) (41) reported that *S. cerevisae* obtained from various production sites accounted for alcoholic fermentation of 'burukutu' produced in Nigeria. The isolation of Candida krusei which is not an alcohol producer from 'burukutu' prepared and sold in Ilorin, Nigeria was reported by Kolawole et al. (2007) (2), while Faparusi et al. (1973) (42) also isolated *Candida kruseifrom* a three day malted grains meant for 'burukutu' production. The production of flavour and aroma has been reported to be associated with the presence of *C. krusei* and that its presence together with non-aflatoxin producing *Aspergillus* spp. could inhibition mycotoxin-producing moulds such as *A. flavus, A. parasiticus* and *Penicilliumi talicum* (43,44).

There was a notable decrease in the aflatoxins content in 'Burukutu' when compared with the substrate (sorghum grains) from where the alcoholic beverage was made. A comparative study between standardized maize-based dishes/snacks and raw maize used for their preparation showed a significant reduction in the aflatoxins content of maize-based dishes/snacks when compared with unfermented substrates (45). Spontaneous fermentation of maize dough for 'Doklu' production was observed to reduce the level of aflatoxins substantially in the raw material (46). Oluwafemi et al. (2010) (17) assessed the ability of LAB in removing AFB1 from maize grains infected with toxigenic *A. flavus*. After 6 days of lactic acid fermentation by *Lactobacillus* spp varied degree of aflatoxin reduction was achieved. More than 50% of aflatoxin B1 reduced in the fermentation initiated by *Lb. casei* and *Lb. acidophilus*, while the mixed-culture of *Lactobacillus* spp gave higher degree of bio-detoxification. Several physical and chemical methods have been exploited for the detoxification or inactivation of mycotoxins in cereals and their derivatives (47,48). However, only a few of these methods have been approved for practical purpose, and there are limitations which are known to reduce their effectiveness. The limitations attached to afore-mentioned methods pave a way for bio-detoxification of aflatoxin by bacterial fermentation and this has been considered to be the best approach (17). The degradation of mycotoxins especially aflatoxins had been reported to be a subject of natural fermentation (22,45,46). It is suggestive that natural fermentation might have reduced the metabolic functions of toxigenic *A. flavus* and the amount of aflatoxins in fermented products. The degradation of aflatoxins and the inhibition of toxigenic *A. flavus* during natural fermentation might be connected with the production of lactic acids by *Lactobacillus* strains and suppressing metabolites released into culture solution (46,49). It has been suggested that removal of aflatoxins during lactic acid fermentation is through non-covalent binding of these toxins to the fractions of the cell wall structure of lactic acid bacteria (50). However, other mechanism involved in complete elimination of aflatoxin B1 has been attributed to LAB fermentation during which lactone ring in aflatoxin B1 is degraded (51). The detoxification of aflatoxins might also be connected to lower pH of the media as other investigations have revealed that the treatment of LAB pellets with acids such as hydrochloric acid significantly promoted the binding ability of the bacteria (46).

5. Conclusion

In conclusion, arrays of fungal isolates associated with 'Burukutu' were revealed in this study. The findings from this study also revealed the aflatoxins bio-detoxification potential of natural fermentation. Spontaneous fermentation is a biological food processing method and it is considered as an effective food-grade bio-preservatives for eliminating food intoxicants of microbial origin in which aflatoxin is one big challenge. The complete bio-detoxification of aflatoxin contamination was not achieved in this study which might be due to the ubiquitous nature of the aflatoxin-producing fungus. The presence of

toxigenic fungi in the samples is of great health issue that must be tackled holistically.

Coflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The technical supports from the Department of Microbiology, Federal University of Technology Akure, Nigeria is highly appreciated.

References

1. Olaniyi OO, Adeleke BS, Akinyele BJ, et al. Microbiological evaluation and antibiotic susceptibility pattern of bacteria associated with 'burukutu', a non-alcoholic beverage. J Food Res Sci 2018; 7: 1-7.
2. Kolawole OM, Kayode RMO, Akinduyo B. Proximate and microbial analyses of 'burukutu' and 'pito' produced in Ilorin, Nigeria. Afric J Biotechnol 2007; 6: 587-90.
3. Eze VC, OkoyeJI, Agwung FD. Chemical and microbiological evaluation of soybeans flours bought from local markets in Onitsha, Anambra State, Nigeria. J Appl Sci 2011; 3: 39-45.
4. Fadahunsi IF, Ogunbanwo ST, Fawole AO, et al. Microbiological and nutritional assessment of 'burukutu' and 'pito' (Indigenous fermented alcoholic beverages in West Africa) during storage. Natural Sci 2013; 11: 98-103.
5. Lynn M, Alibe W, Brisca J, et al. Isolation of some pathogens in 'Burukutu', a local drink sold in Sengere village, Girie local government, Adamawa State. Greener J Microbiol Antimicrob 2014; 2: 1-2.
6. Abbasi F, Liu J, Zhang H, et al.Effects of feeding corn naturally contaminated with aflatoxin on growth performance, apparent ileal digestibility, serum hormones levels and gene expression of Na, KATPase in ducklings. Asian-Austra J Ani Sci 2018; 31: 91–97.
7. Fang J, Zhu P, Yang Z, et al. Selenium ameliorates AFB1-Induced excess apoptosis in chicken splenocytes through death receptor and endoplasmic reticulum pathways. Biol Trace Element Res 2018; 187: 273–83.
8. Guo BJ, Yu C, Holbrook T, et al. Strategies in prevention of pre-harvest aflatoxins contamination in sorghum: Aflatoxin biosynthesis, genetics and genomics. Plant Sci 3: 11-20.
9. Wild CP, Turner PC. The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis 2002; 17: 471-81.
10. Abdallah MF, Girgin G, Baydar T, et al. Occurrence of multiple mycotoxins and otherfungal metabolites in animal feed and maize samples from Egypt using LC-MS/MS. J Sci Food & Agri 2017; 97: 4419- 28.
11. CAST. 2003. Mycotoxins: Risks in plant, animal and human systems. Council Agricultural Science and Technology, Ames, Lowa.
12. Liu N, Wang J, Deng Q, et al. Detoxification of aflatoxin B1 by lactic acid bacteria and hydrated sodium calcium aluminosilicate in broiler chickens. Livestock Sci 2018; 208: 28-32.
13. IARC. Working Group on the Evaluation of Carcinogenic Risks to Humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 2002; 82: 1-556.
14. Saminathan M, Selamat J, Abbasi Pirouz A, et al. Effects of nano-composite adsorbents on the growth performance, serum biochemistry, and organ weights of broilers fed with aflatoxin-contaminated feed. Toxins 2018; 10: 345.
15. The Mycotoxin Factbook: Food and Feed Topics. Barug D, Bhatnagar H, Van Egmond J, Van Der Kamp W, Van Osenbruggen and Visconti A, (Editors). Wageningen Academic Publishers, Wageningen, Netherlands; 2006. P 21-26.
16. Salem R, El-Habashi N, Fadl SE, et al. Effect of probiotic supplement on aflatoxicosisand gene expression in the liver of broiler chicken. Environ Toxicol & Pharmacol 2018; 60: 118-27.
17. Marrez D, Shahy E, El-Sayed H, et al. Detoxification of aflatoxin B1 in milk using lactic acid bacteria. J Biologic Sci 2018; 18: 144-51.
18. Oluwafemi F, Kumar M, Bandyopadhyay R. Bio-detoxification of aflatoxin B1 in artificially contaminated maize grains using lactic acid bacteria. Toxin Reviews 2010; 29: 115-22.
19. Bintvihok A, Kositcharoenkul S, Effect of dietary calcium propionate on performance, hepatic enzymeactivities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon 2006; 47: 41–46.
20. Vijayanandraj S, Brinda R, Kannan K, et al. Detoxification of aflatoxin B1 by an aqueous Microbiological research 2014; 169:294-300.

21. ZinedineA, Faidand M, Benlemlih M, In vitro reduction of aflatoxin B1 by strains of lactic acid bacteriaisolated from Moroccan sourdough bread. Int J Agri & Biol 2005; 7: 67-70.
22. Rayes AAH. Removal of aflatoxin B1 from experimentally contaminated whole milk using a pool of probiotic strains of lactic acid bacteria and baker's yeast *Saccharomyces cerevisiae*. New York Sci J 2013; 6: 84-90.
23. Olayiwola I, Oganah B, Oguntona C, et al. Status of aflatoxin and anti-nutritional contents of standardized maize-based dishes/snacks consumed in Nigeria. J Sci Food & Agri 2013; 1(5): 93-6.
24. Barnett H, Hunter B. 1972. Illustrated genera of imperfect fungi. 3rd ed, Burgess Publishing Company, Minnesota, USA. p23-9.
25. Cocolin L, Aggio D, Manzano M. An application of PCR-DGGE analysis to profile the yeast populations in raw milk. Int Dairy J 2002; 12: 407-11.
26. Dinçkaya E, Kınık O, Sezgintürk M. Development of an impedimetric aflatoxin M1 biosensor based on a DNA probe and gold nanoparticles. Biosens & Bioelectron2011; 26: 3806-11.
27. Jinap S, De Rijk T,Arzandeh S, et al. Aflatoxins determination using in-line immunoaffinity chromatography in foods. Food Control 2011; 26: 42-8.
28. Sobolev V. Simple, rapid and inexpensive cleanup method for quantitation of aflatoxins in important agricultural products by HPLC. J Agri & Food Chem 2007; 55: 2136-41.
29. Ijabadeniyi A. Microorganisms associated with ogi traditionally produced from three varieties of maize. Res J Microbiol 2007; 2: 247-53.
30. Mohammed S, Steenson L, Allen W. Isolation and characterization of microorganisms associated with the traditional sorghum fermentation for production of Sudanese kisra. Appl & Environ Microbiol 1991; 57: 2529-33.
31. Abdel I, Hamad S, Osman M, et al. Characterization and distribution of microorganisms associated with kisra bread preparation from three sorghum varieties in Sudan. Current Res in Bacteriol 2010; 3: 138-47.
32. Oriola O, Boboye B, Adetuyi F. Bacterial and fungal communities associated with the production of a Nigerian fermented beverage, "otika". Jordan J Biologic Sci 2017; 10: 127-33.
33. Efiuvwevwere B, Akoma O. The microbiology of 'kunun-zaki' a cereal beverage from northern Nigeria during the fermentation (production) process. World J Microbiol & Biotechnol 1995; 11: 491-3.
34. Akoma O, Jiya E, Akumka D. Influence of malting on the nutritional characteristics of kunun-zaki. Afric J Biotechnol 2006; 5: 996-1000.
35. Elmahmood A, DoughariJ. Microbial quality assessment of 'kunun-zaki' beverage sold in Gerei town of Adamawa, Nigeria. Afric J Food Sci 2007; 1: 11-15.
36. Adeyemi I, Umar S. Effect of methods of manufacture on quality characteristics of 'Kunun-zaki', a millet based beverage. Niger Food J 1994; 12: 34-40.
37. Bibek R. 2001. Fundamental Food Microbiology, 2st ed. The CRC Press Limited, Washington DC. p56- 90.
38. Akinleye O, FajoluI O, Fasure A, et al. Evaluation of microorganisms at different stages of production of 'ogi' in 'Alimosho' Community, Area Southwest, Lagos, Nigeria. Americ J Res Commun 2014; 2: 215-30.
39. Embashu W. 2014. Physicochemical, nutrient and microbiological analysis of "oshikundu", a cereal based fermented beverage from Namibia. Master of Science Thesis, University of Namibia, Namibia. PhD Thesis; p12-13.
40. Adebayo B, Otunola A, Ajao T. Physiochemical, microbiological and sensory characteristics of 'kunu' prepared from millet, maize and guinea corn and stored at selected temperatures. Adv J Food Sci & Technol 2010; 2: 41-6.
41. Umaru G, Tukur I, Akensire U, et al. Microflora of 'kunun-zaki' and 'zobo' drinks in relation to public health in Jalingo Metropolis, North-East Nigeria Int J food Res 2014; 1:16-21.
42. Achi O. The potentials of upgrading traditionally fermented foods through biotechnology. Afric J Biotechnol 2005; 4: 375-80.
43. Faparusi S, Olofinboba M, Ekundayo J. The Microbiology of 'Burukutu' beer.

ZeitschriftfürallgemeineMikrobiologie 1973; 13:563-8.

44. Halm M, Lillie A, Sorensen A. Microbiological and aromatic characteristics of fermented maize doughs for 'kenkey' production in Ghana. Int J Food Microbiol 1999; 19: 135-43.
45. Oyewole O. Characteristics and significance of yeast involvement in cassava fermentation for "fufu'production. Int J Food Microbiol 2001; 65: 213-8.
46. Ikeorah J, Okoye W. Four decades of research on aflatoxin in Nigeria: A review of NSPRI experience. A paper presented at NAFDAC/IAEA regional workshop on mycotoxin held on the 7th of November, 2005 at Oshodi, Lagos.
47. Marina C, Théodore N, Marina K. Effect of fermentation process on nutritional composition and aflatoxins concentration of 'doklu', a fermented maize based food. Food & Nutr Sci 2013; 4: 1120-7.
48. Chitrangada D, Mishra H. In vitro degradation of aflatoxin B1 by horse raddish peroxidase. Food Chem 2000; 68: 313-6.
49. Galvano F, Piva A, Ritieni A. Dietary strategies to counteract the effects of mycotoxins: a review. J Food Protect 2001; 64: 120-31.
50. Haskard C, El-Nezami H, Kankaanää P. Surface binding of aflatoxin B1 by lactic acid bacteria. Appl & Environ Microbiol 2001; 67: 3086–91.
51. Zhang X, Ohta Y. Binding of mutagens by fractions of the cell wall skeleton of lactic acid bacteria on mutagens. J Dairy Sci 1991; 74: 1477-81.
52. Nout M. Fermented foods and food safety. Food Res Int 1994; 27: 291-8.