



Influence of Different Processing Methods on Aflatoxin Level in *Ogi* Slurry Produced from Millet (*Pennisetum typhoideum*)

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Abstract: The global shortage of cereal foods arising from improper processing and preservation methods adopted in some of our industries could be the sources of contamination of various food products which could lead to devastating health disorder. From this study, millet grains were subjected to different processing methods such as fermentation, alkaline boiling, oven drying and sun drying. The microorganisms associated with the processed samples were isolated and identified using standard microbiological and biochemical methods. The extraction, detection and quantification of aflatoxin from *ogi* slurry were quantified using thin layer chromatography. The sample fermented with ethanol and water showed higher fungal count 37.33cfu/ml while the sample fermented in sodium hypochloride shown no growth of *Aspergillus flavus* with fermentation time at initial inoculation. The milled fermented oven dry sample showed higher colony counts 14.50 cfu/g when compared to milled sun-dry and alkaline boiling fermented samples. The aflatoxin level of the *ogi* slurry ranged from 0.00ppb to 0.02ppb. Samples fermented with water and ethanol showed high effect in removal of aflatoxin B and G produced by toxigenic *Aspergillus flavus* while the alkaline boiling showed less effect on aflatoxin B₁ and B₂. Aflatoxin G₁ and G₂ were completely removed/destroyed from the sample fermented with ordinary water with aflatoxin level 0.00ppb. The sample subjected to oven dry and sun-dry showed complete removal/reduction in aflatoxin B₁ and G₁. The reduction in aflatoxin level of *ogi* slurry produced from millet using different processing methods showed its permissible level below the standard as recommended by National Agency for Food Drug Administration and Control (NAFDAC) and European Union which could be promising prior to this methods in controlling the level of contaminant in our food products for human use.

Keywords: Aflatoxin, *Aspergillus flavus*, Cereals, Food Contamination, *Ogi* Slurry

1. Introduction

Contamination of food products after post-harvesting processing has been the major concern in food industries. This occurrence devalue the wholesomeness of products thereby leading to shortage and reduction in its economic value. The contamination of food might arise from the improper handling of food or during food processing. Food contamination usually results from human, animal, rodents,

insect and microorganisms. Microbial food contamination on cereal products occurs due to suitable environmental condition such as humidity, temperature, pH, moisture content of where they are stored or preserved. Microbial food contamination majorly pose health risk on consumption of such food substance infested with toxic substance called mycotoxin. Mycotoxin are toxin produced from fungi which contaminate both human and animal diets before harvest and during storage thereby causing great economic loss in both the livestock industry and aquaculture [1]. The three major

genera of fungi that produce mycotoxins are, *Aspergillus*, *Fusarium* and *Penicillium* [2]. Though not all mycotoxins are toxic, mostly the mycotoxin produced by *Penicillium notatum* but the toxigenic Aflatoxins strain from *Aspergillus flavus*, *Fusarium* spp on humans results in carcinogenic cellular disruption, cytotoxic necrosis, lose membrane integrity, immunosuppressant, nephrotoxic, neurotoxic and teratogenic [3].

There are different types of aflatoxins produced in nature, Aflatoxin B₁, B₂, G₁, G₂ with derivatives M₁ and M₂ [4]. The most common one is Aflatoxins B₁ which are majorly produced from *Aspergillus flavus* and *Aspergillus parasiticus* [5]. Aflatoxins G₁ and G₂ are produced exclusively by *Aspergillus parasiticus*. The presence of *Aspergillus* spp in food products does not always indicate harmful levels of aflatoxin but with significant risk in consumption of such product [6].

Aflatoxins M₁ and M₂ usually contaminate milk obtained from cows that were fed on moldy grains. These compounds are products of a conversion process in the animal's liver. However, aflatoxin M₁ can be found in the fermentation broth of *Aspergillus parasiticus*. Ingestion of contaminated food by aflatoxins might result in acute toxicity [7]. The infestation of mycotoxins producing fungi could be ameliorated using different processing techniques such as heat treatment, fermentation and chemical treatment of some of the food and food products that are prone to aflatoxin contamination. Therefore, decontamination of our food products by different processes methods from aflatoxin infestation could reduce its toxicity with a view of making safe without mutation through different processing techniques mostly is cereal grains.

2. Materials and Methods

2.1. Fungi Isolate

The toxigenic strains of *Aspergillus flavus* was collected from the Microbiology unit of Food and Science Technology Department, Federal University of Technology, Akure. The toxigenic strains of *Aspergillus flavus* was maintained by inoculation onto a fresh prepared potato dextrose agar. Sterile plunger was used to bore about ten portions of the pure isolate into a vial containing sterile water and two drops of tween 20, labeled and stored in refrigerator until further use.

2.2. Collection of Samples

Seeds of pearl (African) millet were purchased from Oja Oba market, Akure, Ondo State, Nigeria inside a sterile sealable bags and then transported to the Microbiology laboratory for analyses.

2.3. Preparation of Spore Suspension

The freshly prepared potato dextrose agar was inoculated with toxigenic strain of *Aspergillus flavus* then allowed to sporulate at 28±2°C for five days. The spore suspension was harvested and poured into sterile McCartney bottles.

2.4. Preparation of Samples

The weighed 200g of the millet were inoculated with 5.25×10^6 cfu/ml spores of aflatoxigenic strain of *Aspergillus flavus*, steep in water containing ethanol and hypochloride in ratio 1:20 and then ferment for 96 hours. The fermented inoculated millet grains were milled-blended and sieved. The resultant *Ogi* slurry was divided into two portion 50g each. The first portion was boiled in water for 20 minutes. The second portion was added with 0.1M NaOH before boiling (alkaline boiling). About 50.0g portion of *Ogi* slurry was placed in an oven at 105°C for 60 minute and then sun dried for 90 minutes.

2.5. Determination of Total Viable Count of *Aspergillus Flavus*

Serial dilution and pour plate methods were used for determining the total viable counts of toxigenic strain of *Aspergillus flavus* in the samples using appropriate mycological medium. For the isolation, 1.0g of the sample was weighed and macerated, dispensed into 9ml of sterile distilled water and then serially diluted up to 10⁻³ dilution factor. One milliliter from the diluent was pipette into a sterile Petri dish containing 0.02ml of lactic acid, then pour plate with already sterilized molten agar [8].

2.6. Extraction of Aflatoxin from the *Ogi* Slurry

Extraction of Aflatoxin from *Ogi* slurry was determined according to modified method of [9, 10]. Fifty grams (50g) of *Ogi* slurry were put into 500 ml conical flask, 250 ml of methanol:water (60:40 v/v) was added. The flask was placed in a mechanical shaker for 30 minutes. The solution was allowed to settle, filtered using Whatman paper No. 1. One hundred and twenty milliliter (120 ml) of the filtrate was taken into a 250 ml separating funnel, containing 30 ml of saturated sodium chloride solution and 50 ml hexane. The flask was shaken vigorously for 2 minutes and the solutions were allowed to separate. The lower methanol layer was collected in another 250 ml separating funnel and 50 ml chloroform was added. It was then shaken vigorously, giving frequent vents. The chloroform layer was drained into a conical flask containing 5.0g of cupric carbonate. It was shaken and cupric carbonate was allowed to settle. This was filter through Whatman filter paper No. 42 having a bed of anhydrous sodium sulphate. The chloroform extract was then collected in a beaker.

2.7. Detection of Aflatoxin in the *Ogi* Slurry

Cupric carbonate was washed with 25ml chloroform, filter through sodium sulphate bed. The extracts were combined and evaporate in a water bath. The residue was reconstituted by dissolving in 1ml of chloroform and transferred into a screw cap vial. One milliliter (1.0 ml) chloroform and 0.2ml of the reconstituted extract were spotted on a pre-coated 20 × 20 cm thin layer chromatography (TLC) plate along with aflatoxin standard of known concentration. The spotted TLC

plate was developed in an equilibrated tank containing chloroform:acetone (9:1 v/v). The developed TLC plate was air-dried at ambient temperature ($28 \pm 2^\circ\text{C}$) and aflatoxin were detected under UV light at wavelength of 360nm. A colour change from blue to yellow upon exposure to aqueous sulphuric acid (50:50 v/v) confirmed the presence of aflatoxin B₁ [11].

2.8. Quantification of Aflatoxin in the Ogi Slurry

Preparative plates of 0.5 μm thickness were employed for the quantification. Zero point eight milliliter (0.8ml) of stored extracts was applied to the TLC plate for band formation on the chromatograph. The preparative TLC plates were developed in an equilibrated tank in an aflatoxin extraction. The solute rise to the solvent front up to about $\frac{3}{4}$ of the total length of the plate, the plate was taken out of the tank and examined under UV light. Once the area containing the toxin of interest was located after UV light examination, it was scrapped off, washed with chloroform and filtered using Whatman No. 1 filter paper. The extract was evaporated to dryness over a hot water bath and reconstituted with 3ml chloroform. The 3ml reconstituted solution and aflatoxin standard of 20 $\mu\text{g/ml}$ concentration was used to read absorbance on an ultraviolet Spectrophotometer (Cecil

instrument CE505) at a wavelength of 360nm.

2.9. Data Analysis

The data obtained from this study was subjected to analysis of variance (ANOVA) using SPSS Version 16, Window 8 and the mean were compared using Duncan Multiple Range Test.

3. Results

Table 1 shows the total colony counts of toxigenic strain of *Aspergillus flavus* after initial inoculation of the samples. The fungal counts decreased as the inoculation time progresses. The high fungal counts 37.33sfu/ml was recorded at the day 1 of the initial inoculation of sample fermented in ethanol and water, followed by 29.00 sfu/ml at day 2 while the least count 13.00 sfu/ml was obtained at day 4 of the initial inoculation with time. The fungal count of the sample fermented in ordinary water was recorded highest 27.00 sfu/ml at day 1 and the least counts 15.00 sfu/ml was recorded at day 4 with initial inoculation time. The Sample fermented in sodium hypochlorite (H_2PO_4) show no fungal growth throughout the initial inoculation with time.

Table 1. Total colony count of *Aspergillus flavus* after initial inoculation of the samples.

Samples	0 hour	24 hours	48 hours	72 hours
AH (sfu/ml)	27.00 ^a \pm 5.03	17.33 ^b \pm 4.49	18.00 ^b \pm 4.04	15.00 ^b \pm 5.77
AHE (sfu/ml)	37.33 ^a \pm 10.68	29.00 ^a \pm 8.51	27.80 ^a \pm 8.51	13.00 ^b \pm 2.52
AHP (sfu/ml)	0.00 ^c \pm 0.00	0.00 ^c \pm 0.00	0.00 ^c \pm 0.00	0.00 ^c \pm 0.00

Mean values in the same column with the same superscript of alphabet are significantly different at $p \leq 0.05$.

Key: AH= Sample fermented in ordinary water, AHE= Sample fermented in ethanol and water, AHP= Sample fermented in sodium hypochlorite (H_2PO_4).

Table 2. Total colony count of *Aspergillus flavus* after fermented samples had been milled and processed differently.

Samples	No of colonies (cfu/g)
ODT	14.50 ^a \pm 4.28
SDT	0.00 ^b \pm 0.00
ABT	0.00 ^b \pm 0.00

Mean values in the same column with the same superscript of alphabet are significantly different at $p \leq 0.05$.

Key: ODT= Oven dry treatment, SDT= Sun-dry treatment, ABT= Alkaline boiling

Table 2 shows the total colony count of *Aspergillus flavus* after fermented samples had been milled and processed differently. The sample subjected to oven dry treatment had the highest colony count 15.50 sfu/g while the samples subjected to sundry and alkaline boiling showed no colony

growth of 0.00 sfu/g.

Table 3 shows the total spore count after initial inoculation of *Aspergillus flavus* into the samples. The spore count decreased as the fermentation time increases. The sample fermented in ethanol and water recorded the highest spore count when compared to other samples treated. The highest spore count 3.98 sfu/ml was recorded from AHE, at day 1, followed by 3.68 sfu/ml at day 2 while the least spore counts was recorded at day 4 of the fermentation. The highest spore count recorded from sample fermented in ordinary water were not significantly different at day 1 to day 3 while the least spore count 2.45 sfu/ml was obtained at day 4. The samples fermented in water containing sodium hypochlorite (1:20 v/v) show no growth of *Aspergillus flavus* throughout the fermentation period (Table 3).

Table 3. Total spore count after initial inoculation of *Aspergillus flavus* into the samples.

Samples	0 hour	24 hours	48 hours	72 hours
AH (sfu/ml)	3.82 ^a \pm 0.44	3.33 ^a \pm 0.19	3.27 ^a \pm 0.21	2.45 ^b \pm 0.22
AHE (sfu/ml)	3.98 ^a \pm 0.34	3.68 ^a \pm 0.27	3.50 ^a \pm 0.23	2.95 ^b \pm 0.06
AHP (sfu/ml)	0.00 ^c \pm 0.00	0.00 ^c \pm 0.00	0.00 ^c \pm 0.00	0.00 ^c \pm 0.00

Mean values in the same column with the same superscript of alphabet are not significantly different at $P \leq 0.05$.

Key: AH= Sample fermented in ordinary water, AHE= Sample fermented in ethanol and water, AHP= Sample fermented in water and sodium hypochlorite (H_2PO_4).

Table 4 shows the level of aflatoxin (ppb) in processed millet *ogi* slurry. For all the samples, 0.00 ppb aflatoxin level was recorded for AFG₁ µg/kg and AFG₂ µg/kg. The samples fermented in water and ethanol + sun drying showed the highest aflatoxin level 0.03 for AFB₂, followed by samples

fermented in ordinary water and samples fermented in water and ethanol + sun drying for AFB₁. The aflatoxin level 0.01 µg/kg was recorded for samples fermented in water and ethanol and samples fermented in water and ethanol + alkaline boiling for AFB₁ and AFB₂.

Table 4. Level of Aflatoxin (ppb) in processed millet *ogi* slurry.

Sample Code	AFB ₁ µg/kg	AFB ₂ µg/kg	AFG ₁ µg/kg	AFG ₂ µg/kg
1	0.01 ^a ±0.00	0.01 ^a ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00
2	0.02 ^a ±0.00	0.01 ^a ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00
3	0.01 ^a ±0.00	0.01 ^a ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00
4	0.01 ^a ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00
5	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00
6	0.02 ^b ±0.00	0.03 ^b ±0.04	0.01 ^a ±0.00	0.00 ^c ±0.00
7	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00
8	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00

Mean values in the same column with the same superscript of alphabet are not significantly different at $P \leq 0.05$.

Key: AFB₁=Aflatoxin B₁, AFB₂= Aflatoxin B₂, AFG₁= Aflatoxin G₁, AFG₂= Aflatoxin G₂, 1= Samples fermented in water and ethanol, 2= samples fermented in ordinary water, 3= sample 1 + alkaline boiling, 4= sample 2 + alkaline boiling, 5= sample 1 + oven drying, 6= sample 1 + sun drying, 7= sample 2 + oven drying, 8= sample 2 + sun drying.

4. Discussion

The result obtained from this study revealed the different processing techniques adopted reduced the major proportion of aflatoxin in the samples. The reduction in the aflatoxin level of *ogi* slurry treated with sunlight cause a significant decrease in both AFB₁ and total aflatoxins content. The degree of aflatoxin reduction was found to be dependent on the duration of exposure to sunlight. This findings was in agreement with [12] who reported 75% significant ($p < 0.05$) reduction in AFB₁ of the aflatoxin-contaminated crude groundnut oil after solar irradiation treatment for 10 minutes. The solar radiation causes the slight increase in the colour of fatty acid [13]. The results also indicated that solar irradiation was not capable of completely destroying the aflatoxins in the millet and *ogi* samples. This could be due to the homogeneity in the particle size of the millet that would retain a larger proportion of aflatoxins thereby preventing penetration of solar irradiation to the whole sample. Penetration of radiation into aflatoxin recovered from groundnut oil has been reported [14]. The effect of sun drying on samples fermented in water and ethanol was found to be the least effective in degradation of aflatoxins, this could be due to some reactions between ethanol and aflatoxins in the sample that make them resistance to photodegradation.

Previous studies showed that chemical degradation of toxins with chlorine are less satisfactory bisulfate has low efficiency with the possibility of toxic epoxide formation [15]. However, since sodium hypochlorite could effectively eliminate toxigenic strain of *Aspergillus flavus* in 0 hour, grain samples may be steeped in water containing sodium hypochlorite (20:1v/v) for 30 minutes, drained before being steeped in fresh water for the fermentation process. Therefore, the possibility of an epoxide being formed over an extensive period will greatly reduce.

The aflatoxin level of the sample treated with alkaline boiling reduced significantly ($p < 0.05$). Ramzi *et al.* [16] had

reported a reduction in the aflatoxin levels from 127µg/kg in raw maize to 68.6µg/kg in tortillas subjected to alkaline cooking. The result obtained from this study fall with the permissible level of aflatoxin level of most cereals product.

The aflatoxin level of the sample treated with water and water containing ethanol appeared to have some effect in the removal of Aflatoxin B and G in the samples. The the of lactic acid bacteria on the growth of *Aspergillus flavus* has been reported [17]. [18] reported a decrease in aflatoxin content of most food products due to the presence of lactic acid bacteria which inhibit aflatoxin biosynthesis.

Results obtained by various authors are somewhat conflicting, since some of them report partial reduction in some mycotoxins while others note total reduction. From the findings, the mycotoxin levels of millet were relatively high, necessitating stronger processing conditions in terms of alkaline concentration and cooking time.

5. Conclusion

Different processing methods have shown the reduction, decontamination and removal of toxigenic strain of *Aspergillus flavus* that are found to vary in their efficacy in toxin removal from millet and *ogi* samples. 72 hours of fermentation was effective in reduction of aflatoxin level by almost 50%. Photodegradation, oven drying and alkaline boiling were found to be effectively reduced or eliminate Aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂). Sodium hypochlorite was effective at ratio 1:20 (v/v) in removal of toxigenic strain of *Aspergillus flavus* at 0 hour from the millet sample during fermentation.

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