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EFFECTS OF STORAGE TECHNIQUES ON AFLATOXINS LOAD IN MAIZE "OGI" FROM UYO METROPOLIS, AKWA IBOM STATE, NIGERIA

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ABSTRACT

Maize is processed into an uncooked corn starch locally called "ogi" or "akamu" in parts of Nigeria. This work evaluated how different commonly used storage techniques for ogi in Uyo affects its aflatoxins (AFTs) load. Fifty samples of freshly prepared ogi, randomly purchased from four markets in Uyo, were bulked into 1 sample, and thereafter divided into 9 samples of 3 Treatments. Each Treatment represented a storage technique. All samples were screened for AFTs B_1, B_2, G_1 and G_2, using thin layer chromatography (HPTLC) with scanning densitometer[a]. Recovery of toxin and detection limit was >85% and below 1ppb, respectively. A two-way ANOVA of the data was done and means were separated at $p< 0.05$. AFG_1 and AFG_2 were not detected in any of the samples. For AFB_1, Treatments A, B and C had mean values of 3.33±3.33, 28.67 ± 8.01 and 54.33 ± 9.53, respectively, while mean values of AFB_2 in Treatments A, B and C were 0.00 ± 0.00, 3.33 ± 3.33 and 17.00 ± 6.56, respectively. There were significant differences ($p< 0.05$) in the mean values among Treatments. Results from this study suggest that ogi is best consumed fresh and that other commonly used storage techniques can potentially increase AFB_1 and B_2 load in ogi.

Keywords: Corn starch; mycotoxins; spontaneous fermentation; thin layer chromatography.
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INTRODUCTION

Zea mays L., popularly called maize or corn is a member of the family Poaceae (Scot and Emery, 2016). According to Scot and Emery (2016), maize is believed to have originated some 55 – 70 million years ago in the present-day Central or South America and it is currently regarded as one of the main sources of cereals for food, forage and processed industrial products (Kogbe and Adediran, 2003; Dibaba *et al.*, 2013). Maize serves as a staple food providing more than one-third of the calories and proteins in some countries (Kogbe and Adediran, 2003).

In Nigeria, for instance, maize is processed into "ogi" or "akamu", an uncooked corn starch, which is consumed by an estimated 25% of infants and adults every week (Bolaji *et al*., 2015). In Nigeria, Ogi is babies' first cereal and first food after breast milk. It is made from the traditional spontaneous fermentation of white or yellow maize, *Sorghum bicolor* (L.) Moench (Sorghum) or *Pennisetum americanum* (L.) Leeke (Pearl millet) in a decreasing order of preference (Adebukunola *et al*., 2015). Ogi is a staple cereal, found predominantly in Southern Nigeria and is usually also the first native food given to babies at weaning (Onofiok and Nnanyelugo, 1998). It is produced by soaking maize grains in water for 2 to 3 days, during which time spontaneous fermentation takes place. This is followed by wet milling and sieving through a screen mesh. Most mothers, especially in rural and suburban Nigeria, introduce ogi at three to six months of age to their babies (Onofiok and Nnanyelugo, 1998).

Globally, maize grains are known to be prone to mycotoxin contamination, both in the field and in store (Oliveira *et al*., 2017; James and Zikankuba, 2018; Oyeka *et al*., 2019; Garcia-Diaz *et al*., 2020). According to James and Zikankuba (2018), hot humid weather, poor agricultural practices, bad storage conditions and facilities, scanty knowledge of mycotoxins as well as low technical knowhow in the detection of these toxic fungal metabolites in food samples are some of the factors that make Sub-Saharan Africa especially more prone to mycotoxin contamination in food and feed products. Research findings in Sub-Saharan Africa have shown that maize grains are infested by toxigenic fungi (mainly species in the genera *Fusarium* and *Aspergillus*) that contaminate the maize with mycotoxins to varying degree. Mycotoxins often associated with maize include Aflatoxins (AFTs), Deoxynivalenol, Fumonisins, Ochratoxins and Zearalenone (James and Zikankuba, 2018). Of all the mycotoxins, AFTs and fumonisins have been implicated as being the most common (Sanyaolu *et al*., 2019). On the basis of adverse effects on human and animal health and widespread contamination, AFTs are considered the most important mycotoxins on a worldwide scale (Miller, 2008). Bbosa *et al.* (2013) identified 19 types of AFTs thus: Aflatoxin B_1 (AFB_1), Aflatoxin B_2 (AFB_2), Aflatoxin B_{2a} (AFB_{2a}), Aflatoxin B_3 (AFB_3), Afatoxin G_1 (AFG_1), Aflatoxin G_2 (AFG_2). Others are Aflatoxin M_1 (AFM_1) and Aflatoxin M_2 (AFM_2), Aflatoxin M_{2A} (AFM_{2A}), Aflatoxicol (AFL), Aflatoxicol M_1, Aflatoxin G_{2A} (AFG_{2A}), Aflatoxin GM_1 ($AFGM_1$), Aflatoxin GM_2 ($AFGM_2$), Aflatoxin GM_{2A} ($AFGM_{2A}$), Parasiticol (P), Aflatrem, Aspertoxin and Aflatoxin Q_1 (AFQ_1). Their acute and/or chronic effects leading to health problems such as liver cancer, immunosuppression, irritation and respiratory problem, among others, in humans and animals are well documented (Abdel-Wahhab *et al*., 2007; Seo *et al*., 2011; Mehrzad *et al*., 2014 and Sanyaolu *et al*., 2019).

Processing and storage of food rely on a series of technologies that are developed to enhance quality, safety and acceptability (Ross *et al.*, 2002). Fermentation as a food processing technique has been reported to have considerably reduced AFTs load in maize and ogi, respectively (Johnston *et al*., 2012; Adelekan and Nnamah, 2019). The need for further research on the role played by high humidity and temperature on AFTs load in stored maize has also been underscored (Villers, 2014).

There are some literature showing the effect of storage techniques on some important properties of ogi such as its proximate composition, pH, total titrable acidity, pasting characteristics and sensory evaluation (Bolaji *et al*., 2011), as well the effect of storage temperature on the microbiological property and microbial safety of ogi (Inyang and Offiong, 2010). However, there is dearth of information on how the locally preponderant storage techniques of ogi in rural and urban Nigeria affect the AFTs load of this widely consumed food.

Ogi, one of the staple foods of residents in Uyo would be harmful to human health if contaminated by AFTs-producing fungi. Apart from infection of the maize seeds on the field by AFTs-producing fungi, further contamination by these fungi may be caused by unhygienic conditions of the environment during processing, storage and further exposure in the market where this product (Ogi) is sold. The objectives of this work, therefore, were to determine the presence or absence of AFTs in ogi sold in some open markets in Uyo metropolis and to determine how the commonly used storage techniques in Nigeria affect AFTs load in the ogi samples.

MATERIALS AND METHODS

Experimental Samples and Treatments

Fifty samples of freshly prepared ogi/akamu, made using traditional spontaneous fermentation technique, were randomly purchased from 25 sellers from four different open markets namely: Itam, Akpanandem, Usse Offot and Etuk Street, all in Uyo metropolis. These were bulked into 1 sample and thereafter divided into 9 samples comprising 3 different treatments. Each treatment had 3 replicates.

Each of the treatments represented a different storage technique used in Nigeria. Treatment A was freshly prepared ogi stored at room temperature and screened within 24 hours of preparation for AFTs. Treatment B was ogi refrigerated at 4^0C for 7 days before screening for AFTs and Treatment C was ogi submerged under portable water for 7 days before screening (water was decanted and replaced every 24 hours).

Screening for AFTs in Ogi samples

All samples were screened for AFTs B1, B2, G1 and G2 in the Pathology Laboratory of the International Institute of Tropical Agriculture (IITA), Moniya, Ibadan, Oyo State, Nigeria.

Extraction of AFTs from ogi Samples

This was done according to the method described by Bankole and Adebanjo (2003). The extraction was carried out in the following stages: sampling, blending, shaking, filtering, partitioning and dissolving or rinsing. Ogi samples were carefully ground with commercial Waring blender and thoroughly mixed. Thereafter, 20 g of ground sample was weighed out, in 3 replicates, for the purpose of extraction. Each weighed sample was blended with 1000 ml of 70% methanol for three minutes using the Stomacher Laboratory blender (Athena 9 Model). The blended mixture was poured into a 250 ml Pyrex conical flask and sealed with paraffin, after which the samples were shaken using Orbit Shaker at 4 x 100 rmp for 30 minutes. This was thereafter filtered into a clean conical flask (rinsed with methanol) using No. 1 quantitative Whatman filter paper, 185 mm. After this, the filtrate (40 ml) was poured into a separating flask, and 20 ml of distilled water and 25 ml of dichloromethane were added. Again, this mixture was shaken gently and left to separate into top and bottom phases. The extract was drained through a bed of 20 g anhydrous sodium sulphate into a 150 ml white plastic beaker, and 10 ml of dichloromethane was added to this mixture in the separating flask after which it was again shaken gently and allowed to separate. Again, this extract was drained through a bed of 20 g anhydrous sodium sulphate into a 150 ml white plastic beaker containing the first extract, after which the extract was allowed to dry overnight in the fume hood.

Reconstitution of the dried extract

This was done according to the method of Bankole and Adebanjo (2003). Two (2) ml of dicloromethane was added and placed on a vortex mixer to bring up the dried extract from the sample cup and thereafter transferred into 1.5 ml eppendorf tube and allowed to dry in the fume hood for 24 hr.

Quantification of AFTS

Determination and quantification of AFTs were done using thin layer chromatography (TLC) as described by Bankole and Adebanjo (2003). After 24 hr, 1 ml of dichloromethane was dropped into dried extract in eppendorf cup using calibrated dropper and covered. The High Performance Thin Layer Chromatography (HPTLC) was calibrated by 1.5 cm, 7.5 cm, 10 cm from the edge of the paper and cut uniformly. Four (4) ml of the extract was placed on the spot marked 1cm apart and thereafter washed with acetone thrice. The sample was shaken with the

use of vortex mixer at 2500 rpm to mix uniformly after which it was extracted with the use of micro pipette, and the "Aflatoxin mix" was added to the standard track.

Development stage

This was done according to the method of Bankole and Adebanjo (2003), where development solution was prepared with diethyl, ethanol and distilled water in the ratio 96:3:1, respectively. Developing solution was added into the Development chamber and allowed for six (6) minutes. HPTLC plate was inserted into the Development chamber with the calibration facing inside the chamber for about 15-20 minutes after which it was removed and allowed to dry in the fume hood.

Viewing stage

Again, the method of Bankole and Adebanjo (2003) was adopted. The HPTLC plate was placed on CAMAG UV light machine for observation of the aflatoxin levels, after which it was placed on the CAMAG scanner for scanning and reading of the aflatoxin levels. CAMAG scanner reads from 1ppb (part per billion). Aflatoxins level was below 1ppb, and could, therefore, not be detected, even if present. The recovery of toxins was >85%. The scanning densitometer[a] (Camag TLC Scanner 3, ISO 9001, Reg. No. 11668-01) was deployed at this stage.

Analysis of data

All data reported in this study were mean values from 3 replicates. A two-way ANOVA was performed on the data using GraphPad Prism (version 6.01). Means were separated at $p < 0.05$ using LSD (Hamada, 2017)

RESULTS

Table 1 shows that AFTs G_1 and G_2 were below detectable limits in all the treatment samples. For AFB_1 however**,** treatments A, B and C had mean values (ppb) of 3.33±3.33, 28.67 ± 8.01 and 54.33 ± 9.53, respectively, while mean value (ppb) of AFB_2 was 0.00 ± 0.00, 3.33 ± 3.33 and 17.00 ± 6.56 in treatments A, B and C, respectively. There were significant differences ($p < 0.05$) in the mean values among treatments for AFTs B_1 and B_2 (Table 1).

Table 1: Mean AFTs concentration (ppb) in different Ogi samples

Treatment	AFTs concentration (ppb)			
	B1	B2	G1	G2
A	3.33±3.33[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
B	28.67 ± 8.01[b]	3.33 ± 3.33[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
C	54.33 ± 9.53[c]	17.00 ± 6.56[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Mean values with different superscripts in the same column are significantly different at $p<0.05$

DISCUSSION

Whereas AFTs have been described as unavoidable contaminants in foods (Oluwafemi and Ikeowa, 2008), the concern for their presence in food and feeds is a long-standing one, which is global in nature (Motalebi *et al*., 2008; Oyeka *et al*., 2019; Garcia-Diaz *et al*., 2020). These concerns are well placed because of the obvious debilitating dangers in humans and livestock exposed to AFTs poisoning. Literature is replete with the incidence of AFTs contamination in ogi under different circumstances and experimental conditions (Oluwafemi and Ikeowa, 2008; Adegoke *et al*., 2010; Jonathan *et al.*, 2018) because the AFTs could not be completely eliminated from the maize used in producing the ogi (Adegoke *et al*., 2010).

It should be noted that this work reported the presence of AFB_1 in all the 3 treatments and AFB_2 in just 2 of the treatments. Fresh ogi samples (Treatment A) had a mean value of AFB_1 that was less than the maximum permissible range of 4-5 ppb allowed by Nigeria's National Agency for Food, Drugs Administration and Control (NAFDAC), the European Union (EU) and Codex Alimentarius Commission as reported by Makun *et al*. (2010), while AFB_2 was below detection limit in Treatment A samples. This result, therefore, affirms the safety of this important staple when taken fresh, a finding which conforms with the position of Inyang and Offiong (2010). The mean value of AFB_1 in Treatments B and C were higher than the recommended permissible limit, and significantly higher ($p<0.05$) than the mean value of Treatment A; these results raise some concerns on the safety of consuming ogi that is stored for long (7 days and above), either in the refrigerator or using the traditional storage technique. Going by the WHO standard of the safety range of AFTs consumption of between 1-20 ppb as reported by Osuret *et al*. (2016), one may safely conclude that none of the storage techniques resulted in an unsafe AFB_2 level, a fact that cannot be affirmed for AFB_1 with regards to storage for 7 days or more, irrespective of the storage technique used.

Previous studies have affirmed AFTs as being the most widespread and the most debilitating of all mycotoxins (Miller, 2008; Bbosa, 2013). Findings from the present study further reinforce previous report (Bbosa, 2013) that even amongst the AFTs, AFB_1 poses the most concern because it is the most widespread.

The fact that AFG_1 and G_2 were not detected in any of the ogi samples does not suggest that they were altogether absent. The implication of this result is that AFG_1 and G_2 in the samples probably fell below the detectable limit of the machine used in this work. The dangers associated with the persistent consumption of this toxin, especially with regards to the possibility of bioaccumulation, biomagnification and the possibility of potentiation have been highlighted in a previous study by Sanyaolu *et al*. (2019).

In addition, findings from this work validate the position of earlier workers (Villers, 2014; James and Zikankuba, 2018) who reported that apart from field sources, processing and storage techniques, as well as the conditions in the environment at any time can also be veritable routes of contamination of foods by mycotoxins, as was the case with the observed increase reported for AFB_1 and AFB_2 in the ogi samples in storage in this work.

CONCLUSION

Findings from this show that consuming ogi fresh eliminates the dangers of intoxication from AFB_1 and AFB_2, while long-term storage of any sort increases the risk of intoxication by these fungal poisons.

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