Effect of Fermentation on Aflatoxin Concentration in Egusi Local Product (Ogiri)

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Abstract: Ogiri is an important soup condiment processed by fermenting egusi (melon) kernels in Nigeria. *Aspergillus flavus* infected egusi kernels (EK) are often contaminated with carcinogenic aflatoxins. Processing food by fermentation reduces aflatoxin in the end product. However, information on processing egusi to reduce aflatoxin concentration (AC) is limited. Hence, fate of AC in ogiri processed with contaminated EK was investigated. Highly contaminated market and artificially inoculated EK were wetted with sterile distilled water and wrapped with nylon sheets and allowed to ferment (35 days period). Control samples were not fermented. At 7 days interval, samples were collected and analysed for aflatoxin in three replicates using standard analytical methods. After 7 days of fermentation, AC (6.4ng/g) was significantly reduced compared to the control (93.4ng/g) amounting to 93.0% reduction in AC. After a 35 day fermentation period AC was reduced to 2.9ng/g which corresponded to 97.0% reduction. There is significant difference (P = 0.05) between the aflatoxin in the control and the various fermented samples. Artificially inoculated samples gave similar results. After 7 days of fermentation, aflatoxin was 515.0ng/g while control was 11011.3ng/ resulting to 49% reduction of aflatoxin. Concentrations of aflatoxin reduced with increase in the fermentation period. This trend continued as the days progressed. After 35 days fermentation, aflatoxin was 57.7ng/g, (94% reduction). Fermentation of contaminated EK reduced aflatoxin contamination. Consumption of fermented egusi (ogiri) can reduce risk of aflatoxin exposure since up to 90% reduction can be obtained.

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Introduction

Colocynthis citrullus L. (melon) is an important food crop in many sub-Saharan African countries. It is commonly called egusi in Nigeria. It is produced in abundance in many parts of Nigeria especially the central regions, Nassarawa and Niger states in particular (Van der Vossen *et al*., 2004). In West Africa, it is called 'egusi'. In Nigeria, it is cultivated as an increasingly important cash crop. Melon is easy to grow in Nigeria's warm climate during the beginning of the rainy season and harvested at the onset of the dry season (Van der Vossen *et al*., 2004; Brisibe *et al.*, 2011).

The white, bitter flesh of the melon is not edible, but the seeds are a staple of many local diet. The seed is also roasted and eaten as snacks (Van der Vossen *et al*., 2004). In some parts of eastern Nigeria, the unshelled seeds are fried and served in the burial ceremony of aged women to commemorate their old age. It is processed into a variety of forms in different regions of Africa. In Kalahari region of Namibia, the seeds after roasting are ground into a coarse, whitish meal, which is nutritious and pleasantly nutty-tasting. In West Africa the seeds are made into pulp and added as a thickener to soups. They are soaked, boiled, fermented and wrapped in leaves to form a favourite local food seasoning called 'ogiri' or they are roasted, pounded, wrapped in leaves and then boiled to produce another sweetener called 'igbalo' (Okeke *et al*., 2008; Oluba *et al*., 2008; Aboloma and Ogunbusola, 2012). The pulp of roasted and salted seeds is eaten in Sudan and Egypt, where it is called 'tasali'. In the far northern parts of Sudan seeds of some types are processed to 'gorom' by roasting the unshelled seeds which are eaten whole. Melon as an oil seed, a highly prized vegetable oil extracted from the seed is used for cooking (Ayodele and Salami, 2006). The residue from oil extraction is made into balls that are fried to produce a local snack called 'robo' in Nigeria. The seeds can be roasted to make a substitute for coffee. The seed is rich in oil and protein and contains good quantities of most of the essential amino acids (Ayodele and Salami, 2006).

The composition of dried melon seed without shell per 100 g is 5.1 g water, 2340 kJ energy, 28.3 g protein, 47.4 g fat 15.3 g carbohydrate, 54 mg Ca, 755 mg P, 7.3 mg Fe, 0.19 mg thiamin, 0.15 mg riboflavin, 3.55 mg niacin and 58 µg folate. The oil is clear, semi-drying and easily refinable (Ayodele and Salami, 2006).

Melon kernels have been reported to be contaminated with aflatoxins as a result of infection by toxigenic *Aspergillus flavus* (Somorin and Bankole, 2010). Aflatoxins are naturally occurring mycotoxins which are produced by many species of the fungus *Aspergillus*, most notably *Aspergillus flavus* (Link) and *Aspergillus parasiticus* (Speare). Aflatoxins are produced as a result of the metabolic activities of toxin-producing *Aspergillus* in infected crop species (Othman and AL-Delamiy, 2012). Aflatoxins are among the most carcinogenic substances known (IARC, 2002). In addition to being potent hepatotoxic and carcinogenic metabolites, aflatoxins also affect child growth and development and adversely affect immune status (Wild, 2007). Aflatoxins are quite stable in many food processes, resistant to degradation and are not destroyed under normal cooking conditions (https://ntp.niehs.nih.gov/ntp/roc/content/profiles/aflatoxins.pdf). Many factors such as the presence of protein, pH, temperature and length of treatment affect effectiveness of some processing methods in reducing concentrations of aflatoxins in food can be affected by many (Fandohan *et al.,* 2005,2008; http://www.ehso.com/ehshome/aflatoxin.php). Aflatoxins decompose at their melting points, which are 237°C for aflatoxin G1, 264°C for aflatoxin B and 299°C for M1. Processing food by fermentation has been reported to remove aflatoxins in them. This is advantageous because it is a mild method of food processing, which preserves the nutritive value and flavour of de-contaminated food. Laboratory fermentation has been shown over the years to irreversibly degrade mycotoxins without leaving any toxic residues (Biernasiak et *al*., 2006). The detoxifying effect of fermentation is through toxin binding effect (Egwim *et al.,* 2013). Fermentation degrades aflatoxin by opening up the aflatoxin B1 lactone ring leading to its complete detoxification (Nout, 1994; Assohoun *et al.,* 2013).

The fermentation of melon seed for "ogiri" production can substantially reduce the amount of aflatoxins contaminating the raw material (Ogunsanwo *et al.,* 1989. The level of aflatoxins found in the processed melon products are much below those of the raw seeds (Bankole *et al.,* 2010). The fermentation of melon seeds could have accounted for the low level of aflatoxin reported in melon products by Bankole *et al.* (2010) in their study compared to levels reported in unfermented melon seeds (Biernasiak *et al*., 2006; Fandohan *et al.* 2008). The consumption of this fermented product of melon can reduce the risk of aflatoxin exposure since considerable aflatoxin reduction can be obtained by fermentation. This study was undertaken to determine the effect of fermentation on aflatoxin concentration in melon local product (ogiri) commonly used as a seasoning in many parts of Nigeria.

1. Materials and methods

Melon kernels from a local market Oja Oba in Osun State that were highly contaminated with aflatoxin were collected. The contaminated melon kernels were fermented by wrapping them with nylon sheets after wetting with sterile distilled water. Also, clean melon kernels were artificially inoculated a toxigenic strain of *Aspergillus flavus* and incubated for 5 days; thereafter it was fermented as described above. The control samples were not fermentation. At 7 days interval, samples were collected and analysed to determine the aflatoxin concentration for up to 5 weeks in three replicates.

Aflatoxin was extracted using the modifications of Bankole *et al.* (2004), Countryman *et al*. (2009) and Odoemelam and Osu (2009) and quantified following the methods described by Aquino *et al*. (2005), Atehnkeng *et al*. (2008b), and Leslie *et al*. (2008).

Percentage aflatoxin reduction was calculated using the formula:

$$\text{Percentage reduction} = 100 - \frac{\text{Treated}-\text{control}}{\text{Control}} \times 100$$

1.1. Data analysis

Data on aflatoxin concentration generated were summarized and analyzed using analysis of variance of the SAS (version 9.1, SAS Institute, Cary, NC). Means were compared using least significant difference test (LSD) at P = 0.05 procedure in SAS (SAS Institute Inc., Cary, NC, USA) to compare the differences among the samples fermented for different length of days.

2. Results

The results of the effect of fermentation on aflatoxin concentration of contaminated melon kernels are presented in Figures 1 and 2 below. After 7 days of fermentation, the aflatoxin concentration was 515.0ng/g while the control (unfermented egusi) was 11011.3 ng/g amounting to 49 % reduction of aflatoxin. There was significant difference (P = 0.05) between the aflatoxin in the control and the various fermented samples. The concentrations of aflatoxin in the samples were shown to reduce with increase in the fermentation period. This trend continued as the days progressed. At 14th and 21st day after fermentation aflatoxin concentrations was 352.2 and 313 ng/g respectively with corresponding aflatoxin reductions of 65 and 69 % which did not differ significantly. After a 35 day fermentation period aflatoxin concentration was 57.7 ng/g, which resulted to 94.0% reduction in aflatoxin concentration. However, this was not significantly different from aflatoxin

concentration after 28 days fermentation (72.7 ng/g) which reduced aflatoxin concentration by 92.3% (Figure 1).

The ogiri made from marker samples gave similar results. Afte 7 days of fermentation, the aflatoxin concentration (6.4 ng/g) was significantly reduced compared to the control (93.4 ng/g) giving rise to 93 % reduction in aflatoxin concentration. This trend continued as the days progressed. After a 35 day melon kernel fermentation period aflatoxin concentration was reduced to 2.9 ng/g which corresponded to 97 % reduction in aflatoxin concentration. The second experiment was better because after 7 days of fermentation there was over 90% reduction in aflatoxin concentration and thereafter no significant differences was observed in aflatoxin concentration between the 7 days fermentation period and the other days (Figure 2).

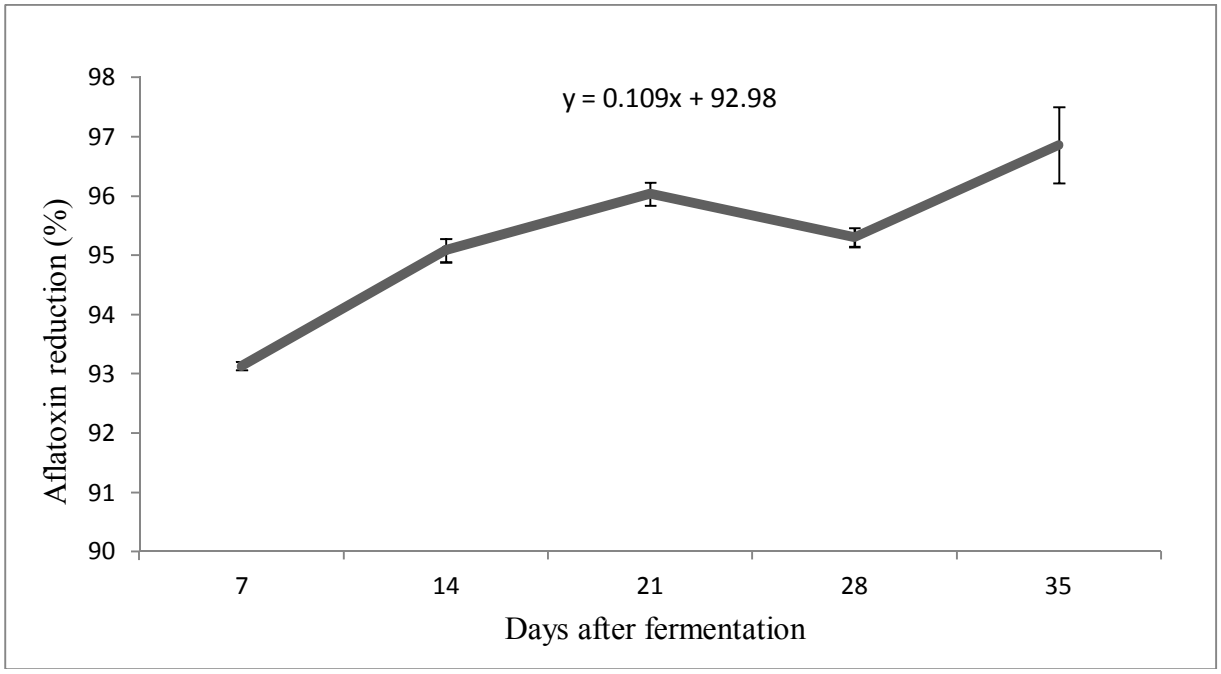

Figure 1. Effect of fermentation on aflatoxin concentration in inoculated melon kernel

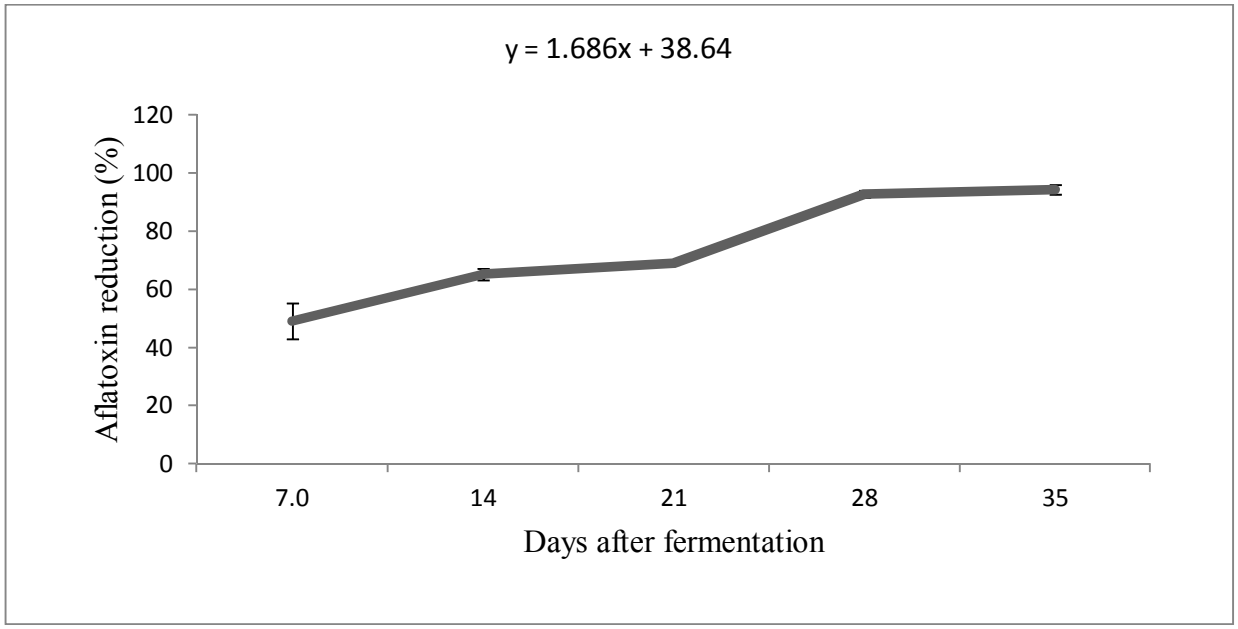

Figure 2. Effect of fermentation on aflatoxin concentration in naturally contaminated

3. Discussions

Fermentation improves the nutrient content of the foods being fermented. Processing food by fermentation for detoxification is advantageous because it is a milder method, which preserves the nutritive value and flavour of decontaminated food. In addition to this, fermentation irreversibly degrades mycotoxins without leaving any toxic residues. Kim (2007) reported reduction of growth and aflatoxin production by an aflatoxigenic mold in the presence of bacteria which are found in fermented foods.

Laboratory fermentation has been demonstrated over the years (Biernasiak et *al.*, 2006). The detoxifying effect is believed to be through toxin binding effect (Egwim *et al.,* 2013). The aflatoxin content of both artificially and naturally contaminated melon kernel after processing to the local condiment (ogiri) was significantly reduced after the fermentation process. A similar observation has been reported in artificially inoculated melon seed used for the production of "ogiri" (Ogunsanwo *et al.,* 1989). This agrees with the report by Bankole *et al.* (2010), which the levels of aflatoxin found in processed melon seeds are much below those of the raw seeds.

This study has shown that natural fermentation of melon seed for "ogiri" production can substantially reduce the amount of aflatoxins contaminating geusi kernel. Similarly, Jasutiene et al. (2007) and Montaseri et al. (2014) reported decrease in the concentration of aflatoxin M_1 in milk fermented for yorghurt production. Fermentation of melon can help to reduce aflatoxin concentration in contaminated seeds. The fermentation of melon kernels could have accounted for the low level of occurrence of aflatoxin observed in this study and by the authors cited above. Fermentation has been reported as alternative mechanism of aflatoxin removal which opens up the aflatoxin B1 lactone ring resulting in its complete detoxification (Nout, 1994; Assohoun *et al.,* 2013). Fandohan *et al.* (2008) also reported that percentage loss of aflatoxins in food is variable depending on the techniques involved in processing it.

The significant reduction in the levels of the toxins observed during fermentation could be as a result of the microbial activity and effect of pH which are inter-woven (Ogunsanwo *et al.,* 1989). Furthermore, competition for substrates by the microbes could also be a factor in the degradation of the aflatoxin in "ogiri". Ogueke *et al.* (2010) reported that fermentation decreases the number of *Aspergillus* species colonies with increase in the number of days of fermentation of ugba (fermented African oil bean Seeds)**.** Thus fermentation creates an environment that is not suitable for their growth and toxin production, especially with the increasing pH of the fermenting foods into the alkaline region.

Fermentation of contaminated melon kernels recorded aflatoxin reduction of 49 – 94% and 90 - 97 % in the first and second experiments, respectively. Fermentation is a mild method that offers various advantages, including, improved food safety, nutritional value, enhanced flavour and acceptability, reduction in anti-nutrients, detoxification of toxigenic compounds, and enhanced shelf life of the product. The consumption of this fermented product of melon can reduce the risk of aflatoxin exposure since up to 90% reduction can be obtained by fermentation.

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