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Fermentation of Aflatoxin Contaminated White Dent Maize (*Zea mays*)

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Abstract: White dent maize was sorted manually into apparently clean and visibly spoilt maize samples. The maize samples were pulverized and fermented for Ogi production. The clean and spoilt maize samples had 11.8 and 18.6% moisture content, respectively. During fermentation, the concentration of aflatoxin B present in the apparently clean white dent maize slurry rose from 0.98 μg mL^{-1} at day 0-1.05 μg mL^{-1} at day 1 while for visibly spoilt white dent maize slurry the concentration rose from 2.20 μg mL^{-1} at day 0-2.55 μg mL^{-1} at day 1. The study also shows the persistence of aflatoxin B throughout the fermentation period with concentrations remaining at 1.05 and 2.55 μg mL^{-1}, respectively for both samples. Fermentation was accompanied with decrease in pH of the slurry from 6.0 at day 0-4.0 at day 3 for apparently clean white dent maize slurry and 5.8 at day 0-4.0 at day 3 for visibly spoilt white dent maize slurry. There was a corresponding increase in the titratable acidity from 0.2 at day 0-1.1 at day 3 and 0.4 at day 0-2.3 at day 3, respectively. This study shows a deleterious health hazard in the continuous use of aflatoxin B contaminated maize in Ogi production.

Key words: Aflatoxin, aflatoxigenic, persistence, fermentation, maize, production

INTRODUCTION

Fermentation is one of the oldest and most economical methods of processing and preserving foods (Chauvan and Kadan, 1989). A fermented food is one with one or more of its constituents acted upon by microorganisms to produce a considerably altered final product acceptable to human use (Van Veen and Steinkraus, 1970).

Fermented foods provide varieties in the diet (Kordylas, 1990), increase water-soluble vitamins (Van Veen and Steinkraus, 1970) and improve organoleptic properties and digestibility (Chauvan and Kadan, 1989).

In developing countries like Nigeria, fermentation techniques are passed on as trade secrets in the families of certain communities, a practice protected by tradition. Moreover, fermented foods constitute a significant component of the diets mostly in the rural areas. The traditional food processing system which involves use of maize is diversified and a large variety of foods are produced and sold by food processing micro enterprises. The high temperature and high humidity coupled with unsanitary conditions in tropical countries favored food spoilage and pathogenic microorganisms (Nout and Rombouts, 1992).

Aflatoxins are one of the most potent toxic substances that occur naturally. They are produced by *Aspergillus flavus* and *A, parasiticus* (Polster *et al*., 1990). They are classified as a group one carcinogen and have been confirmed as a potential carcinogen in agricultural product (IARC, 1993). Metabolism plays a major role in deciding the degree of toxicity (Eaton and Gallagher, 1994).

Aflatoxin 8, 9 epoxide, one of the metabolites of aflatoxin metabolism can induce mutation by intercalating into DNA by forming adduct with guanine moiety in the DNA (Smela and Sophie, 2001). Moreover, species susceptibility to aflatoxin is mainly dependent on its liver detoxification system, genetic make-up, age, environmental factor, exposure level and nutritional status of diet (Howard and David, 1990).

Various studies have shown statistical relationship between hepatoma and consumption of aflatoxin (Neto, 1981) as found in Swaziland (Keen and Martin, 1971), Uganda (Alpert *et al*., 1971), Kenya (Peers and Linsell, 1973); Mozambique (Van Ransburg *et al*., 1975), Nigeria (Bababunmi *et al*., 1977) and exposure and incidence of liver cancer (Groopman and Wild, 2001). Aflatoxin is relatively stable to heat (Rehana and Bassapa, 1990). Ugali and bread prepared from contaminated maize and wheat retained 85.5 and 82.4% aflatoxin B and G, respectively in Ugali while the bread retained 83.8 and 67.8% aflatoxin B and G, respectively (Seenappa and Nyagahungu, 1983). Undoubtedly, there is a pertinent need to prevent aflatoxin production. However, maize grains cannot be free of fungal propagules, hence any attempt to control

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the occurrence of mycotoxins requires the development of efficient analytical procedure so that the levels of consumption in foods may be accurately monitored. This study tends to establish the food safety and possible health hazard as it relates to the possibility of the presence of aflatoxin in fermenting maize.

MATERIALS AND METHODS

Source of maize: Maize samples used in this study were commercial low moisture white dent bought from Emir's market in Ilorin, Kwara State, Nigeria.

Sorting of maize: Maize grains were manually separated in to clean and visibly spoilt maize manually based on visual examination. Physical damage, decay, insect injury, discoloration, moldy appearance, chipped and broken defined visibly spoilt maize.

Moisture level determination: About 80 g of each samples of the sorted maize grains were weighed in to aluminum dish and heated in a Gallenkamp oven Bs model ov-330 at 105°C and weighed periodically until constant weight was obtained (AOAC, 1984). The percentage moisture present was calculated with the formula:

$$\text{Moisture (\%)} = \frac{\text{Weight of water loss}}{\text{Weight of maize before drying}} \times 100$$

Typing for aflatoxin presence in maize samples: About 50 g of each maize sample were weighed in to sterile 500 mL conical flask. About 100 mL of chloroform was added to each conical flask and shaken vigorously on orbital shaker at 120 rpm for 20 min. The chloroform extract was filtered through dried No. 1 Whatman filter paper.

Clean up of extract: Basic green cupric carbonate powder was added to the crude chloroform extract at the rate of 10 g 100 mL^{-1} chloroform extract (Alozie *et al*., 1980) to remove yellow pigment. Excess cupric carbonate and cupric carbonate bound yellow pigment were separated by filtration using dried No. 1 Whatman filter paper.

Analysis of aflatoxin

Qualitative analysis: The clear chloroform extract of each maize sample was evaporated to dryness in a rotary flask evaporator. The residue of each maize sample extract was redissolved in 2 mL of reagent grade chloroform. Aliquot of each maize sample extract was subjected to thin layer chromatography on silica gel plates by spotting the aliquot approximately 4 cm from the bottom of the 20×20×0.3 cm chromatographic plate (Anon, 1975). The spots were placed 2 cm apart and the plate was developed to a distance of 15 cm in an unlined developing tank containing chloroform: acetone (9:1) solvent system. The plate was dried at room temperature and later observed under ultraviolet light using the chromato-vue cabinet at 365 nm. The patterns of fluorescent spots were observed and the colour noted with the R_f.

Quantitative: This was done by the spectrophotometric method of Roderick and Stoloff (1970). The concentration of the two aflatoxin extracts prepared by redisolving the aflatoxin spot from the silica gel in chloroform was determined by measuring the absorbance (A) with a spectrophotometer (SP8-150 uv/vis PYE UNICAM) at 365 nm against chloroform as the blank. The concentration of each type of aflatoxin was calculated with the formula:

$$\text{Conc. of aflatoxin } \mu g\ mL^{-1} = \frac{A \times MW \times 100 \times CF}{E}$$

Where:

A = Optical density
MW = Molecular weight
E = Molar absorptivity
CF = Correlation factor (obtained from calibration of instrument)

The molecular and molar absorptivity were taken from the table provided by Roderick and Stoloff (1970).

Confirmation of aflatoxin: The characteristic fluorescence properties and R_f value provided confirmation of the presence of the presumptive aflatoxin as observed under ultraviolet light at 365 nm. The chromatographic plate was also sprayed with 10% H_2S0_4 in methanol. A bright yellow spot also confirmed aflatoxin (Romer, 1975). Finally, the chloroform extract was derivatized withTrifluoroacetic Acid (TFA) to convert the naturally occurring aflatoxin to more intensely fluorescent hemiacetals (AOAC, 1984). These features were compared with a know aflatoxin standard.

Fermentation of maize samples: About 200 g of pulverized samples of the apparently clean maize and visibly spoilt maize were introduced in to separate clean sterile 1000 mL conical flask. About 400 mL of sterile tap water was added into each conical flask. The two flasks were stoppered with cotton wool and incubated at room temperature 27±2°C for 3 days for the maize to ferment.

Determination of pH and titratable acidity: The pH of the maize slurry was measured with a PYE unicam pH meter (292 MK 2) at interval of 24 h until the end of the fermentation process. The method of Kirk and Sawyer (1991) was used to determine titratable acidity.

Aflatoxin behavior during fermentation: About 40 mL of water and 0.6 g NaCl was added to 30 mL of each slurry sample in a 500 mL conical flask. The flask was shaken vigorously in an orbital shaker at 120 rpm for 10 min. About 40 mL of the flask content was added to 7 mL ethanol and 20 mL hexane in a 500 mL conical flask. The content was again shaken in an orbital shaker for 4 h at 120 rpm. Centrifuging at 2000 rpm for 5 min separated the methanol water extract. Adding 25 mL of 17% NaCl solution to 50 mL methanol extract diluted the methanol extract. About 75 mL of the resultant solution was washed with 50 mL chloroform in a separating funnel and subjected to analyses for aflatoxin.

RESULTS AND DISCUSSION

Moisture level determination: The average moisture level of the apparently clean white dent maize was found to be 11.8% while that of the visibly spoilt white dent maize was found to be 18.6%.

Determination of pH: The pH changes that occurred during the fermentation of white dent maize sample slurries are shown in Fig. 1a. There was a decrease in the pH from day 0-3 in both maize sample slurries. For apparently clean white dent maize slurry it decreased from 6.0 at day 0-4.0 at day 3 while for visibly spoilt white dent maize slurry it decreased from 5.8 at day 0-4.0 at day 3 (Fig. 1a).

Determination of titratable acidity: The result of titratable acidity carried out during fermentation of white dent maize slurries are shown in Fig. 1b. There was an increase in the titratable acidity from day 0-3 day for both maize samples. For apparently clean white dent maize slurry it increased from 0.2 at day 0-1.1 at day 3 while that of the visibly spoilt white dent maize slurry increased from 0.4 at day 0-2.3 at day 3 (Fig. 1b).

Behavior of aflatoxin B during fermentation of maize: The concentration of aflatoxin B in the white dent maize slurries of the apparently clean and visibly spoilt maize samples were monitored from day 0 of fermentation through day 3 as shown in Fig. 2. There was a slight increase in the concentration of the toxin in both maize samples from day 0-1 day. For the apparently clean white dent maize slurry, the aflatoxin B concentration rose from 0.98 $\mu g\ mL^{-1}$ at day 0-1.05 $\mu g\ mL^{-1}$ at day 1, while for visibly spoilt white dent maize slurry the concentration rose from 2.2 $\mu g\ mL^{-1}$ at day 0-2.55 $\mu g\ mL^{-1}$ at day 1

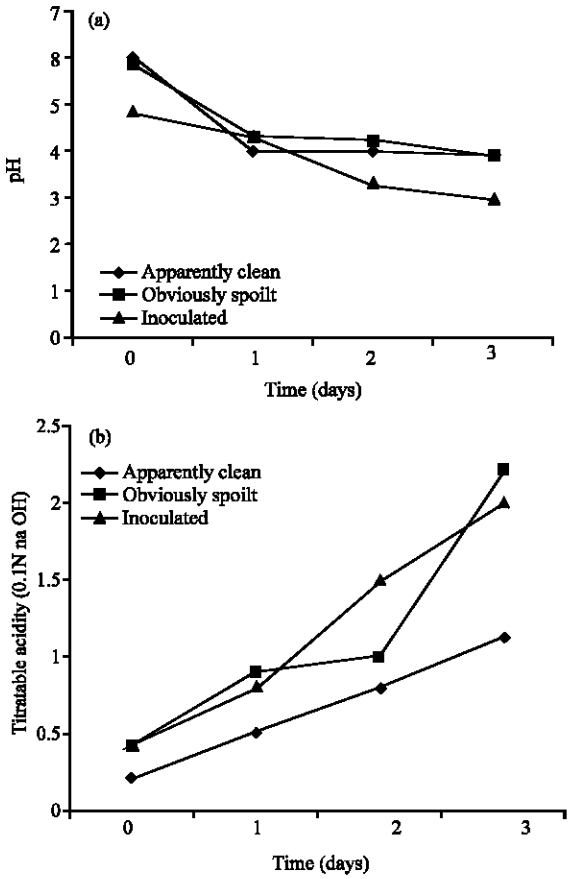

Fig. 1: (a) Changes in pH of white dent maize slurry during fermentation (b) Changes in titratable acidity of white dent maize slurry during fermentation

(Fig. 2). The concentration of the toxin then persisted through to day 3 during the 3 day fermentation. The average moisture level of the apparently clean maize sample 11.8% was smaller than that of the visibly spoilt maize sample 18.6%.

This could be as a result of those factors which defined spoilt grains which have exposed the moisture absorbent internal surfaces of the endosperm of the visibly spoilt white dent maize grains (Adegoke and Adeyemi, 2004). The increased physiological activity of the spoilage organisms in the visibly spoilt maize may also be associated with high moisture. Temperature and moisture are the two most important factor governing growth and toxin production in agricultural commodity.

The minimum moisture level for aflatoxin production depends on the nature of substrate and duration of storage (Janardhana *et al.*, 1992). The sharp drop in the pH as the fermentation progressed from day 0-3 (Fig. la) and a corresponding increase in the titratable acidity (Fig. 1b) was consistent with the result in the fermentation

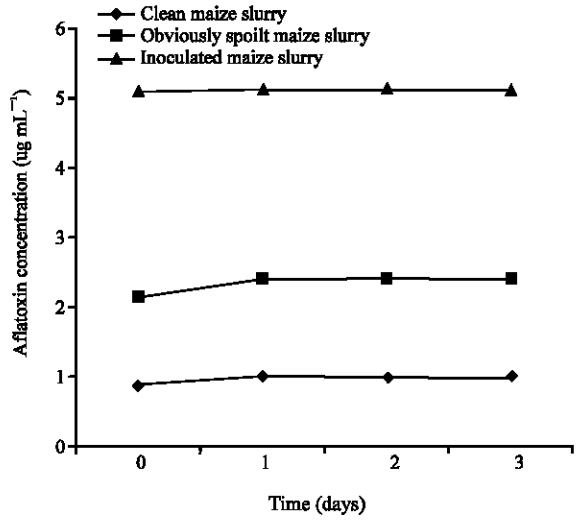

Fig. 2: Changes in concentration of aflatoxin in white dent maize slurry during fermentation

of the traditional Ghanaian corn dough (Mensah *et al*., 1991) and the Nigeria Ogi (Adegoke and Adeyemi, 2004). However, these changes do not have any effect on the stability of the aflatoxin B present in the fermenting maize as the concentration of the toxin persisted throughout the fermentation period (Fig. 2).

The pertinent need that led to this study was the fact that it has been established that method of preparation and processing of food may account for detoxification (Sall *et al*., 1988) and destruction of aflatoxin (Mishra and Daradhinger, 1991).

Hence, the study was fashioned to monitor the effect of fermentation, on aflatoxin of maize. Diet is the major way through which humans as well as animals are exposed to aflatoxins. However, occupational exposures in agricultural workers, people working in oil milks and graneries have been reported (Sorenson *et al*., 1984).

Considering Fig. 2, aflatoxin B was relatively stable to maize fermentation. The concentration rose in the maize samples from day 0-1 probably due to increased libration of the pre-formed aflatoxin in to the medium. However, further increment stopped after day 1 and the already produced aflatoxin remained in the fermentation medium for both maize samples. Studies on the stability of aflatoxin during brewing indicated that it is fairly stable to heat although, some are lost during wort boiling and mashing (Chu *et al*., 1975). This has a serious health implication since the already formed aflatoxin remained in the medium, being stable to maize fermentation. This implies that if aflatoxin contaminated maize is used in Ogi production, it is not destroyed by the fermentation process. Aflatoxin being relatively stable to heat (Rehana and Bassapa, 1990) means that the hot water used to gelatinize Ogi may not inactivate or destroy the aflatoxin, substantially as cooking which entails boiling over prolonged period, caused only 41% maximum inactivation of cooked port (Furtado *et al*., 1981).

CONCLUSION

Consequently, fermented maize foods prepared from aflatoxin contaminated maize are left at the mercy of method of preparation and processing to determine the salability, wholesomeness and safety. The United Nation Protein Advisory Group accept a maximum level of 30 μg kg^{-1} of aflatoxin in food supplements for undernourished children in developing countries (Uraih and Ugbadu, 1980) while the German Government permitted levels of aflatoxin in milk for infant is 50 ng kg^{-1} and for dietetic it is 10 ng kg^{-1}, 1991). The concentration of aflatoxin present in these maize slurry samples are high and this study shows that there is an elevated risk of aflatoxicoses in the current and potential use of aflatoxin contaminated maize at village level in Nigeria and suggests the need for control of overall mould contamination of maize grains.

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