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Fate of Aflatoxin Levels in Cereals and Cereal Products During Processing

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Abstract: The fate of aflatoxin during processing of contaminated maize grains into "guguru (roasted maize/popcorn) at different temperature regimes and at different time intervals showed that aflatoxin can be destroyed with heat. Gradual reductions in aflatoxin B1 levels as processing temperature and time increased were observed. Processing temperatures of 100°C, 150°C, 200°C, and 250°C with 30 minutes time intervals for a maximum period of 2 hours were used. Reduction of aflatoxin (99.5%) was achieved with grains processed at 250°C for 30 minutes. The other levels of reductions were from 20% at 100°C for 30 minutes to maximum of 97.6% reduction at 200 ℃ for 120 minutes. The concentration of aflatoxin in the raw materials used for brewing showed that all materials such as sorghum, corn grit, barley, even the fungicides had low levels of aflatoxin. The cumulative effects of aflatoxin in the raw materials caused an increase in the aflatoxin level to about 5.20µg\kg. Boiling the wort at 100°C gave 10.4% e reduction. Further reduction of aflatoxin to 0.450µg/kg was achieved during lagering. The pH before and after lagering was 5.2 and 3.7 respectively

Key words: Aflatoxin, temperature, time, maize grains

Introduction

Three cereal grains which constitute the majority of the cereals grown in Nigeria are maize, sorghum (guinea corn) and millet (Oyenuga, 1968). Though maize (*Zea mays* Linn) is predominantly the cereal crop of southern Nigeria, while sorghum and millet are those of the North.

Traditional food products obtained fro0m these cereal grains include boiled maize-on-cob "langbe" roasted shelled and winnower maize grains in a sandy medium yield "Guguru" can be further converted through dry-milling with or without frying to either: aadun" or tanfirin". Other products from the three cereals include "fura gero" "fura ukpo oka "kokoro" (Oguntunde, 1989). Being seasonal crops, especially in West Africa, maize, millet and sorghum are stored as dry grains and from an enormous reserve of food. However, substantial amounts in storage are subject to attack by a variety of insects, fungi and other biodeteriorating agents.

Aflatoxins have been found in many agricultural commodities including maize (Aja and Emejuaiwe, 1994). The incidence of aflatoxins in foods is relatively high in tropical and sub-tropical regions where the warm humid weather provides optimal conditions for growth of the moulds – *Aspergillus flarus* and *A. parasiticus*. With increasing knowledge and awareness of aflatoxins as a potent source of health hazards to both man and farm animals, a great deal of effort has been made to completely eliminated the toxin or reduced its content in foods to significant lower levels.

The import of reducing the aflatoxin content through processing cannot be ignored since contamination of foods by aflatoxin is often unavoidable and still remains a serious problem associated many agricultural commodities.

Aflatoxins are highly toxic, mutagenic and carcinogenic compounds that have been implicated as causative agents in human hepatic and extrahepatic carcinogenesis (Massey *et al.* 1995). The unusual disability of aflatoxins in the agricultural commodities raises the question of whether they could be carried through the food process. Generally processing of foods may expose the food to conditions, which may favour mould development or reduce level of aflatoxin in the final products.

Ingestion of aflatoxins lead to substantial loss of productivity and degrading of meat quality in farm animals consuming contaminated feeds (Bonomi *et al* 1993, 1994). Economic loss for example in 1996, there was an estimated loss of about 140 million U.S. dollars as a directs sequence of weight loss in broilers consuming low levels of mycotoxins (Palmgren and Hayes, 1987).

A large proportion of the Nigerian population depends on maize, millet and sorghum as the principal sources of food. Indeed as many as 18 different kinds of foods can be prepared from these cereals (Oguntunde, 1989). However, aflatoxin being an adventitious toxic contaminant present in cereals and cereal products continues to be a serious problem to the food industry. This work was designed with aim of determining appropriate conditions for the destruction of aflatoxins in some cereal-based foods/drinks

Materials and Methods

Roasted Maize (guguru): A set of 14 trays with each tray containing 100g of contaminated maize grains bought from a local market in Nigeria were all subjected to temperature regimes of 100-200°C for a time interval of 30minutes up to 120mins. The 13th tray containing contaminated maize grains was subjected only to 250°c for 30mins. The 14th tray-the control sample was not subjected to heating.

All maize grains roasted to 'guguru' were milled into fine powder into fine powder using a hammer mill and the moisture contents of all samples were determined using the oven dry method (OAAC, 1975). Tests were done in duplicate.

Aflatoxin detection and measurement: Aflatoxin detection was carried out with 5g of sample. To this amount of sample

was added 50ml of chloroform and 5ml of water and the mixtures were shaken for 30minutes. The filtrate obtained with Whatman No.1 filter paper was evaporated to dryness in a water bath. After evaporation of chloroform from the extract, the extract was redissolved in 1ml of chloroform and then chromatographed on a TLC glass plate (20 by 20cm). The developing solvent was diethyl ether-chloroform-water (95:4:1). The spots were visualized at 366 using a UV light. Quantification of aflatoxin B_1 was done by comparison of the peak heights with those of known standard (Durakovic *et al.*,1985).

Lager beer production and detection of aflatoxin along the line of production (club lager beer)

Duplicate samples were analyzed for aflatoxin levels and pH values at five stages during the brewing process

i. The completed cooker mash (wort)
ii. The combined mash at the end of carbohydrate hydrolysis stage.
iii. The wort after the addition of hops and boiling
iv. Lager beer and
v. Bottled beer after pasteurization (at 63°C for 30minutes)

For the raw materials, 5g of milled samples were used for the extraction and for the liquid samples, 50ml aliquot was used. To the solid samples, 50ml of chloroform and 5ml of distilled water were added. To the liquid samples, 50ml of chloroform was added. All samples were agitated for 30minutes. The solid samples were filtered using Whatman No.1 filter paper. The chloroform layer in the liquid samples was obtained using a separating funnel and the procedure was repeated thrice. the extracts were pooled together and evaporated to dryness in a water bath and redissolved in 1ml of chloroform. Aflatoxin B_1 was then identified by thin-layer chromatography on precoated silica gel plates. Good separation was achieved with the solvent system: diethyl ether-chloroform-water (95:4:1). The spots were visualized at 366nm using a UV light. Quantification of aflatoxin B_1 was done by comparison of the peak heights against those of known standard (Durakovic *et al.* 1985).

Statistical Analysis: All samples were analyzed using Dunca's Multiple Range Test and analysis of variance.

Results and Discussion

Effects of heating on the aflatoxin level of contaminated maize grains are shown in Fig. 1-3. Heating at 100°C for 30 minutes gave aflatoxin values higher than untreated sample. However, as the heating time increased 120 minutes, there was a reduction in the aflatoxin level from 600ug/kg to 384µg/kg and the moisture content of the grains was 7.6%. Heating at 150°C followed the same pattern of heating at 100°C. Heating at 200°C for 30 and 60 minutes showed that there was no difference in aflatoxin B1 levels from the control sample. When heating time was increased to 120minutes, there was drastic reduction of aflatoxin from 600µg/kg (Fig. 3). When heating temperature was increased to 250°C for a period of 30minutes the grains were charred, percentage reduction of aflatoxin was as high as 99.5%.

Fewell (1966), found isolated aflatoxin to be stable up to their melting points of about 250°C. According to Betina, (1989), aflatoxin have high decomposition temperatures ranging from 237°C to 306°C. Solid aflatoxin B1 (AFB1) is quite stable to dry heating at temperatures below thermal decomposition temperature of 267°C. Heating at 100°C, 150°C for 120 minutes and 90 minutes respectively that give high values of aflatoxin could be due to sampling problem because normal home cooking conditions such boiling and frying (approx. 150°C) failed to destroy AFB1 and AFG1 in the Solid State (Kamimura, 1989).

Temperatures above 150°C were necessary to attain partial destruction of the toxin as heating at 200°C for 120minutes reduced the AFB! Level 600µg/kg to 14.4µg/kg. At 250°C for 30minutes, there was almost complete destruction of aflatoxin in the contaminated maize grain. The extent of the destruction achieved was very dependent on the initial level of contamination, heating temperature and time. Degradation of aflatoxin by heat is also governed by the moisture

Table 1: Percentage reduction of aflatoxin levels in maize grains heated between 100-250°C

Temperature	Time (min)	Moisture	AFB_1 (µg/kg)	% Reduction
-	-	116	600	-
100°C	30	9.6	720	20
	60	8.6	384	36
	90	8.2	600	0
	120	7.6	384	36
150°C	30	9.4	600	0
	60	8.4	720	20
	90	8.2	840	40
	120	7.6	384	36
200°C	30	6.0	600	0
	60	6.0	600	0
	90	5.8	540	10
	120	5.4	14.4	97.6
250°C	30	2	3	99.0

Table 2: Change in pH and aflatoxin B_1 levels along the line of beer production

Sample	PH	Aflatoxin B_1 (µg/kg)
Corn	-	0
Sorghum	-	3.4
Barley	-	1.4
Fungamyl	-	0.075
Thremamyl	-	0.350
Sugar	-	0
Wort	5.2	3.8
Wort + Yeast	5.0	3.7
Bright bottle beer	3.8	1.0
Lager beer	3.7	0.45
Pasteurized beer	3.6	1.0

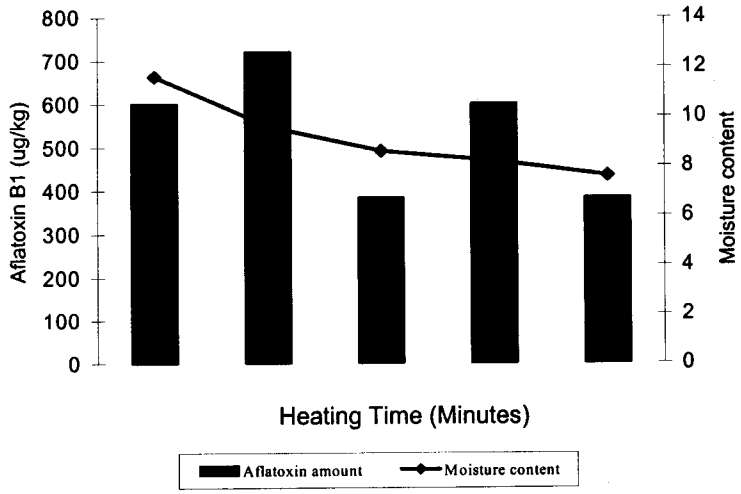

Fig. 1: Effects of heating aflatoxin contaminated maize at a constant temperature of 100°C at different time intervals during the production of "guguru"

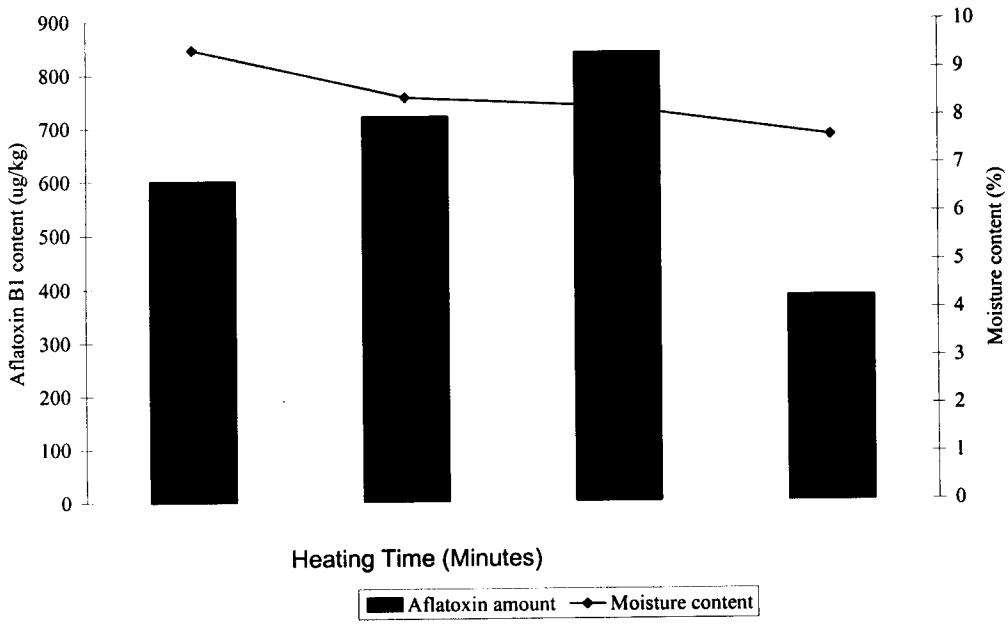

Fig. 2: Effects of heating aflatoxin contaminated maize at a constant temperature of 150°C at different time intervals during the production of "guguru"

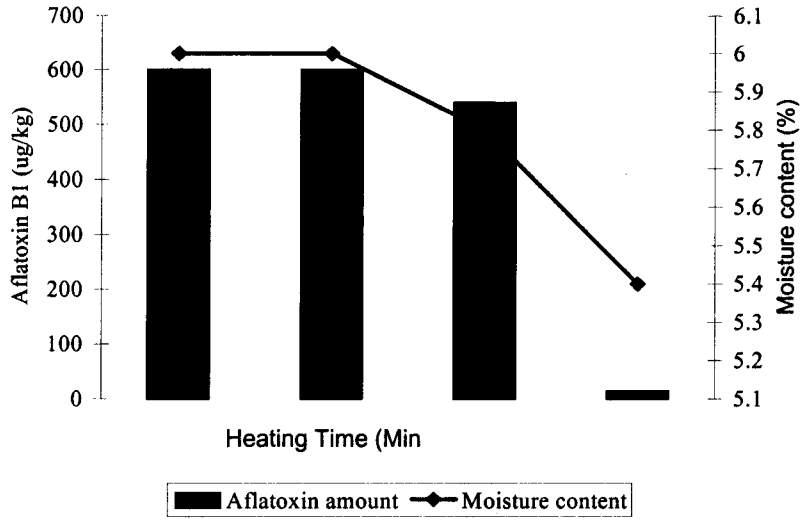

Fig. 3: Effects of heating aflatoxin contaminated maize at a constant temperature of 200°C at different time intervals during the production of "guguru"

content, pH and ionic strength of the food. The moisture content is a critical factor; contaminated foods that contain more moisture can more easily be inactivated by heat. One major reason why high temperatures employed in this study could not effectively detoxity aflatoxin was due to the low moisture content of the grains (11.6%). Mann *et al.* (1967) observed that heating a cottonseed meal containing 30% moisture at 100°C for 1hour degraded 74.8% of aflatoxin (B1+B2) present in the meal whereas in this present work heating at 100°C for 120minutes with a moisture of 11.6% gave only 33.4% reduction. It has been suggested that the presence of water help in opening the lactone ring in AFB1 by the addition of a water molecule to the ring to form a terminal carboxylic acid. The terminal acid group thereafter undergoes heat-induced decarboxylation (Coomes *et al.*, 1966).

Another processing method employed in this study was fermentation. The aflatoxin B1 content of the raw materials used in the brewing process ranged from zero to 3.4µg/kg with sorghum grains having the highest aflatoxins level. The aflatoxin level in work at pH 5.2 was 3.8µg/kg and represented a 10.4% reduction from the initial level of 5.225µg/kg. (Table 2).

When yeast (*Saccharomyces carisbergensis)* was added to the wort the pH feel to 5.0 and the level of AFB1 was 3.7µg/kg. However, after the yeast was filtered off, a pH of 3.8 was recorded and the aflatoxin level was as low as 1.0µg/kg. After period of 30-40 days of maturation, the pH fell to 3.7 giving aflatoxin B1 level of 0.45µg/kg and after pasteurization (63°C for 30mins) at a pH 3.6, the final aflatoxin B1 level in the final product was 1.0µg/kg (Table 2). Thus in general, aflatoxin B1 was destroyed during brewing. Since aflatoxin is relatively stable to heat, it is not surprising that more than 90% of aflatoxin was in the sample after cooker mash treatment. However, removal of aflatoxin from the product at lagering stage was very significant (with a loss of about 89%). The mechanism of aflatoxin removal could be due to the pH reduction (from pH 5.2 to 3.7) coupled with non-specific interaction or absorption of aflatoxin by solid particles removed by filtration process (Chu *et al*, 1975). AFB1 removed from the beer was quite significant (89%).

Conclusion

The hazardous nature of aflatoxin to humans and animals ha necessitated the need for establishment control measures. No doubt consideration of preventive measures aimed at reducing infestation of agricultural commodities with aflatoxin-genic moulds is the best way to control contamination with aflatoxin. Despite all precaution aflation contamination still occurs since the moulds are ubiquitous. Therefore detoxication methods such as heat and fermentation processes when employed can go along way in reducing the levels of toxic aflatoxin to less toxic doses.

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