Research Journal of Food and Nutrition
Volume 1, Issue 1, 2017, PP 23-28

Aflatoxins and Microbiological Characteristics of Stored Bread fruit (Artocarpus Communis) Chips Treated with Aframomum Danielli and Sodium Metabisulphite

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Received Date: 27-10-2017 ***Accepted Date: 02-11-2017*** ***Published Date: 29-11-2017***

ABSTRACT

Breadfruit is a valuable food resource with significant amounts of certainminerals, vitamins and other essential amino acids. Wholesome fresh breadfruit was processed into dried chips and treated with ground Aframomum danielli and sodium metabisulphite salt in the same proportions. The treated breadfruit chips were packaged in polyethylene bags and transparent buckets at different storage conditions. The total aflatoxins of the chips using high performance liquid chromatography were found to be 0.0 – 2.43ug / kg and microbial loads ranged between 0.2to 2.9x 10^2 cfu/g and 0.0 to 1.0 x10^4 cfu/g. The study showed that properly dried and stored breadfruit chipscan be free from or have minimal aflatoxins and microbial loads with or without pre-treatment.

Keywords: *Breadfruit chips, Aflatoxins,Aframomum danielli, Microbial loads, Sodium metabisulphite*

INTRODUCTION

Breadfruit (*Artocarpus communis)* is the fruit of a tropical tree of the family Moraceae. It is essentially a carbohydrate source, eaten in Nigeria, almost exclusively around the region where it is produced. It has been found to be of superior nutritive value than conventional sources of calories like yam, cocoyam, and cassava (Singh, 2009). Breadfruit in the fresh form is highly perishable (Amusa *et al.*, 2002) and long term storage for shipment under commercial condition is not feasible at the present stage of technical development (Medlicott, 2002). Steve *et al.*, 1995 reported that 60-80% of breadfruit produced in South-West Nigeria is wasted as a result of deterioration and lack of use. In Barbados, flour made from the dried fruit is sometimes partly substituted for wheat flour in bread making and found more nutritious than wheat flour in lysine and other essential amino acids (SPORE, 2007). Due to its high amount of carbohydrate, it can easily replace carbohydrate- rich fruit like banana, though it is hydrolysable, but its carbohydrate is thought to be higher (Ajani *et al*.,2012 ;Oladunjoye *et al*.,2010).

Aflatoxins are a group of polyketide mycotoxins that are produced mainly by members of the genus *Aspergillus*. Production of these toxic secondary metabolites is closely related to fungal developmentwhich commonly contaminates food grains before and after harvest.(Keller *et al*.,2005; Jamali *et al*., 2012).These fungi are common and wide spread in nature they occur in warm climates, principally in soil, decaying vegetation, hay and grains undergoing microbiological deterioration and invade all types of organic substrates whenever conditions are favourable for its growth. The toxicity of aflatoxins was recognized in the 1960s and it was later appreciated as a health problem for domestic animals and humans (Razzaghi-Abyaneh *et al*., 2013). Aflatoxin was detected in 98% of samples of dried yam chips surveyed in Benin with levels ranging from 22 to 220µg/ kg and a mean value of 14µg/kg *(Bassa et al*, 2001). Aflatoxin B_1 was detected in 22% of yam chips in some states of Nigeria (Bankole and

Mabejoke, 2003), while Odoemelam and Osu (2009) also found aflatoxin B_1 (40.06-48.59µg/kg) concentrations in breadfruit bought in the market. The continuing threat by aflatoxin contamination of food, feed and agricultural commodities to the world populationare important in food safety, therefore there is need to determine and reduce the levels of these toxic metabolites in stored breadfruit chips under different treatments, storage condition and different packaging materials so as to increase its safety.

MATERIALS AND METHODS

Materials

Procurement Of Raw Materials

Matured green ripe and wholesome fruits of breadfruit (*Artocarpus communis*) were obtained from farmer`s market in Ile-ife, Osun State. *Aframomum danielli* (Family-Zingiberaceae) was bought from Bodija market in Ibadan. The fruits and all other ingredients were taken to Nigerian Stored Products Research Institute, Ibadan processing centre for immediate use in a clean polythene bags.

Processing of Breadfruit into Chips

Wholesome fresh breadfruit were thoroughly washed, peeled and sliced manually into 1cm thick using stainless steel knives. The breadfruit chips were divided into six equal portions (5kg).Some samples were dipped inside 5% solution of sodium metabisulphite while others in*Aframomum danielli* at same proportion. Breadfruit chips were drained and dry inside a cabinet dryer (Nigerian Stored Product Research Institute multi-purpose dryer) at 55°C for 16hours. Samples were packaged inside the polythene bags and transparent buckets with cover for further use. The flow chart is as shown in figure 1.

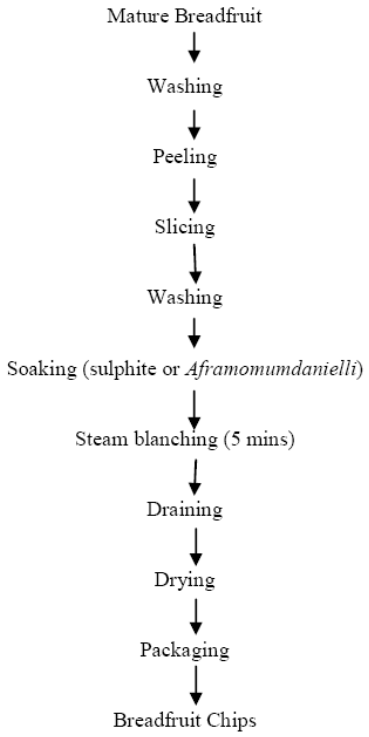

Figure1. *Production of Breadfruit Chips*

Analyses

Aflatoxin determination of the breadfruit chips were done using high performance liquid chromatography (AOAC, 2005).The samples were cleaned up with aflastar immune affinity column 1000; detection was done using Hitachi HPLC with fluorescence detector after post column derivatization using kobra cell. 25g of test sample was extracted with150ml methanol: water (80:20), the extract was filtered through what man #4 filter paper. 20ml of the extract was diluted with 120ml phosphate buffered saline (PBS). 50ml of the diluted extract was cleaned up with Romer aflastar immune affinity column, after a wash step, the aflatoxins were eluted with 1.5ml HPLC methanol and 1ml of the eluate was diluted with 2ml HPLC water. 99ul of the solution was analyzed and quantified by HPLC with fluorescence detection xex = 333nm and 82m = 460nm.

Microbiological analyses were carried out as described elsewhere (Olutiola *et al*. 1991).

RESULTS AND DISCUSSION

The result of the total Aflatoxins of the dried breadfruit samples are as presented in Table 1. Samples D, E, ABU and BBU did not have *Aspergillus flavus* after 3months of storage. Other samples like A, B, C, F, CBU, DBU, EBU with *A. Flatus* had Aflatoxins levels that were below the LOD (least value for detection) according to Codex Alimentarius Commission. This means that the chips were not infected with Aflatoxins and are safe for consumption. The observation is similar to the results obtained by Arowora *et al*. (2012) who stored dried breadfruit chips for 12 months. Although, Odoemelam and Osu (2009) reported the concentrations of Aflatoxins B1 (40.06-48.59 ug/kg) from breadfruit purchased from the market elsewhere, this could be as a result of climatic condition during harvesting, handling, processing or packaging. Thus, differences in harvest and storage conditions as well as the agricultural practices (in each location) also influenced the level of Aflatoxins contamination of the commodities. Medina *et al*. (2014)established the potential impact of key environmental factors, such as water activity (a_w), temperature and atmospheric CO_2, and their interactions on ecology, growth and aflatoxin production by the *A. flavus* both *in vitro* and on maize.

Table1. *HPLC Analysis of Breadfruit Chips after Three Months of Storage*

	Test Performed	**Result**	**Expected**
A	Total Aflatoxin (ug/kg)	Below LOD, LOD = 1.76ug/kg	10.0ug/kg Max
B	"	"	"
C	"	"	"
D	"	None Detected	10.0ug/kg Max
E	"	None Detected	10.0ug/kg Max
F	"	Below LOD, LOD = 1.76ug/kg	"
ABU	"	None Detected	"
BBU	"	None Detected	"
CBU	"	Below LOD, LOD = 1.76ug/kg	"
DBU	"	"	"
EBU	"	"	"
FBU	"	2.43ug/kg	10.0ug/kg Max

LOD: Least value for detection.

The European Countries as well as Codex Committee proposed maximum limit of Aflatoxins for foodstuffs, to be 4 parts per billion in cereals, edible nuts and dried fruit to 10ppb for nuts that will be subjected to further processing (FAO, 2003) while USFDA action guideline is 20 ppb total Aflatoxins for all products intended for feed or food. However, the permitted level of Aflatoxins in food products by the World Health Organization (WHO) is 0 ppb for children, 20 ppb for adults and 55 ppb for animals.

Table2.*Fungal Counts of Breadfruit Chips after One Month of Storage*

*Sample	**Total Fungal Count** (10^2(cfu/g))	**Total Fungal Count** (10^4 (cfu/g))
A	0.8×10^2	Nil
B	0.6×10^2	Nil
C	0.4×10^2	Nil
D	0.4×10^2	Nil
E	0.2×10^2	Nil
F	2.9×10^2	1.0×10^4
ABU	0.2×10^2	Nil
BBU	Nil	Nil
CBU	0.2×10^2	Nil
DBU	0.4×10^2	Nil
EBU	0.6×10^2	Nil
FBU	0.2×10^2	Nil

**A: dried breadfruit alone packaged inside the polythene bag at ambient temperature.*

B: blanched dried breadfruit packaged inside polythene bag at ambient temperature.

C: blanched dried breadfruit with sodium meta bisulphate solution packaged inside polythene bag at ambient temperature.

D: blanched dried breadfruit with Aframomumdanielli solution packaged inside the polythene bag at ambient temperature.

E: dried breadfruit soaked with Aframomumdanielli solution packaged inside the polythene bag at ambient temperature.

F: dried breadfruit soaked with sodium Meta bisulphate solution packaged inside the polythene bag at ambient temperature.

ABU: dried breadfruit without sodium Meta bisulphate and Aframomumdanielli packaged inside the transparent bucket at ambient temperature.

BBU: blanched dried breadfruit without sodium meta bisulphate and Aframomumdanielli packaged inside the transparent bucket at ambient temperature.

CBU: blanched dried breadfruit with sodium meta bisulphate solution packaged inside the transparent bucket at ambient temperature.

DBU: blanched dried breadfruit with Aframomumdanielli solution packaged inside transparent bucket at ambient temperature.

EBU: dried breadfruit soaked with Aframomumdanielli solution packaged inside the transparent bucket at ambient temperature.

FBU: dried breadfruit soaked with sodium meta bisulphate solution packaged inside the transparent bucket at ambient temperature.

The fungal counts of samples examined in this study (Table 2) ranged from 0.2 to 2.9 $x10^2$ c fu/g and 0.0 to 1.0 x 10^4 c fu/g while some samples showed no fungal growth. While the amount of *Aspergillus* spp. present in food is not necessarily indicative of the level of aflatoxin production (Smart *et al*., 1990), presence of some fungi in food samples can be of importance from the standpoint of food safety (Adegoke, 2004).The increased concern in the microbiological safety of food has led to the use of several methods, including artificial and non-chemical preservatives to prevent or reduce the incidence in food commodities. *Aframomum danielli* has been reported to be a good antioxidant and have good inhibitory properties on *Aspergillus spp,* aflatoxi genic moulds and on food spoilage yeasts (Adegoke *et al.,* 2015; Jatto *et al.*, 2010).*Aspergillus flavus* is the most common producer of Aflatoxins and it is a contaminant in agricultural products (Muthoni *et al*, 2009) and host crops are particularly susceptible to infection by *Aspergillusspp*. after prolonged exposure to a high humidity environment or crops are damaged from stressful conditions like drought.

Furthermore, from the results obtained, Lueck (1980) noted the efficacy and antimicrobial activities of sodium Meta bisulphate. Ashaye *et al*. (2006) also, reported that some preservative constituents of *A. danielli* might have disrupted the aflatoxin biosynthetic pathway of which resulted to low production in relation to *A. flavus* growth in samples examined. The effectiveness of low concentration of *A. danielli* extracts is in agreement with the inhibition of the spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice *A. danielli* extract at low concentration (Adegoke *et al*., 2000).Hence, control of Aflatoxins contamination through usage of *Aframomumdanielli* and sodium metabisulphite might provide the greatest reduction in contamination.

Table3.*Characteristics of Dried Breadfruit Chips Stored in Polythene Nylon at Ambient Temperature*

Sample	Appearance	Colour	Crispiness	Odour	Overall Acceptability
A	3.90 ± 0.9	4.00 ± 0.8	3.70 ± 0.7	4.00 ± 0.8	4.00 ± 0.5
B	3.50 ± 1.1	3.90 ± 0.9	3.70 ± 1.0	3.80 ± 0.8	3.60 ± 0.8
C	3.40 ± 1.4	3.80 ± 1.0	4.20 ± 0.9	4.00 ± 0.7	3.20 ± 0.8
D	4.00 ± 0.9	4.00 ± 0.9	4.30 ± 0.7	3.90 ± 0.9	3.90 ± 0.7
E	4.30 ± 1.0	4.20 ± 1.0	4.40 ± 0.7	4.50 ± 0.5	4.00 ± 1.2
F	4.70 ± 0.5	4.80 ± 0.4	4.30 ± 0.8	4.40 ± 0.7	4.70 ± 0.5

Values in the same column not followed by the same letter are significantly different at 5% level of significance.

A-Dried breadfruit alone

B-Blanched dried breadfruit

C-Blanched dried breadfruit with sodium metabisulphite solution

D- Blanched dried breadfruit with Aframomum danielli solution

E- Dried breadfruit with Aframomum danielli solution

F- Dried breadfruit with sodium metabisulphite solution

Table4. *Characteristics of Dried Bread fruit Chips Stored in Transparent Bucket at Ambient Temperature.*

Sample	Appearance	Colour	Crispiness	Odour	Overall Acceptability
A	$4.40^{ab} \pm 0.8$	$4.10^{ab} \pm 0.7$	$3.50^{ab} \pm 0.9$	$4.50^{b} \pm 0.5$	$4.20^{ab} \pm 0.6$
B	$4.20^{a} \pm 0.6$	$3.60^{a} \pm 0.7$	$3.80^{ab} \pm 0.9$	$2.80^{a} \pm 1.3$	$3.60^{a} \pm 0.7$
C	$4.00^{a} \pm 0.7$	$3.70^{a} \pm 1.0$	$3.90^{ab} \pm 0.6$	$2.70^{a} \pm 1.2$	$3.80^{a} \pm 0.9$
D	$4.10^{a} \pm 0.6$	$4.00^{a} \pm 0.7$	$3.30^{a} \pm 0.7$	$3.20^{a} \pm 0.9$	$3.50^{a} \pm 0.7$
E	$4.80^{b} \pm 0.4$	$4.70^{b} \pm 0.5$	$4.20^{b} \pm 0.9$	$4.40^{b} \pm 0.8$	$4.50^{b} \pm 0.8$
F	$4.90^{b} \pm 0.3$	$4.70^{b} \pm 0.5$	$4.00^{ab} \pm 0.9$	$4.70^{b} \pm 0.5$	$4.80^{c} \pm 0.4$

Values in the same column not followed by the same letter are significantly different at 5% level of significance.

A-Dried breadfruit alone

B-Blanched dried breadfruit

C-Blanched dried breadfruit with sodium metabisulphite solution

D- Blanched dried breadfruit with Aframomum danielli solution

E- Dried breadfruit with Aframomum danielli solution

F- Dried breadfruit with sodium metabisulphite solution

The results of sensory evaluation for the breadfruit chips are as shown in Table 3 and 4respectively. The results showed that sample F(dried breadfruit soaked with sodium metabisulphite solution packaged inside the polythene bag and transparent bucket at ambient temperature) of the two packaging treatments was the most preferred in terms of appearance, color, crispiness and odor followed by sample A and E. Table 3 and 4 shown little or no difference in all the samples at 5% level of significance with packaging at the ambient temperature. Also, there is neither insect infestation nor foreign attacks in all the samples. Some of the chips maintained the creamy color and smell naturally without so much alteration in acceptability. All the same, critical control points need to be monitored during processing and it is advisable to maintain good personal hygiene by the handlers at all time to ensure consumers safety.

CONCLUSION

When it is realized that food research has been directed towards diversification in several developing countries of the world, it must be noted that breadfruit has immense potential if processed under good hygienic condition. This study has shown that Aflatoxins and microbial loads in breadfruit chips are minimal thus some processing techniques that can be used to reduce human exposure to Aflatoxins, like cleaning to remove poor quality breadfruit samples and thorough washing before processing, drying breadfruit chips to a safe moisture level and storing in an air tight container are useful processes that can be adopted to prevent post-harvest losses.

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