IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 11 Ver. I (Nov. 2016), PP 10-15
www.iosrjournals.org

Anti-aflatoxigenic Effect of Aframomum danielli on Peanut Balls (Kulikuli)

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Abstract: *The anti-aflatoxigenic effect of Afromomum daniellion peanut balls (kulikuli) and the acceptability of the product were studied. A total of nine (9) samples including the raw material were analyzed using four different Aframomum danielli levels. Four of thesamples were produced without the peanut skin and four were produced with the peanut skin. Peanut ball samples were treatedwith 0, 25, 50 and 100ppm of Afromomum daniellipowder. Proximate analysis of the samples was done and the Kinetics of total aflatoxin reduction was quantified using enzyme-linked immunosorbent assay (ELISA). Sensory evaluations of the samples were also done. Moisture contents of the treated samples ranged from 0.45 to 2.20% and with raw sample having the highest value of 9.25%. Crude protein of Kulikuli ranged between 23.4% and 25.67%. All the samples examined had detectable levels of aflatoxin, ranging from 0.7µg/kg to 3.3µg/kg for the processed samples. While 5.8µg/kg of aflatoxin was detected on the raw peanut. Aflatoxin reduction efficiency of Aframomum daniellifrom raw material to the processed peanut balls ranges between 43.1% and 87.9% while for the finished product but untreated to treated ranges from 70.8% to 12.5%. Organoleptic attributes evaluation of the samples was done, samples treated with the low spice levels irrespective of either with the skin or without the skin were generally accepted by the panelists in terms of colour, taste, flavor and crunchiness.*

Keywords: *Peanuts, Peanut balls,kulikuli, Aframomumdanielli, anti-aflatoxigenicagent, aflatoxin*

I. Introduction

Peanut or groundnut (*Arachis hypogeal*) is a species in the legume family Fabacae which is native to South America, Mexico and Central America (Seijo, *et al*., 2008). It is an annual plant growing to 30cm to 50 cm (1 to 1½ ft) tall. It is grown throughout the tropical and warm temperature regions of the World, (Putnam *et al*., 1991). Peanut is an important oil and food crop and it is the third major oilseed of the world next to soybean and cotton, it also serves as a dietary protein sources for a large segment of the low income populace. (Aletor and Ojelabi, 2007., FAO, 1995). Edible peanuts or the peanut grown primarily for human consumption has several uses as whole seeds or in its processed form. Popular confections include salted peanuts, peanut butter, peanut brittle and shelled nuts (plain or roasted) (Yao, 2004). Salted peanuts are usually roasted and in retail size, plastic ba0gs, dry roasted, salted peanuts are also marketed in significant quantities. Boiled peanuts are a preparation of raw, unshelled fresh peanuts boiled with salt. Another important product of peanut is peanut ball (*Kulikuli*) which is a shelled, roasted, milled, deoiled, spiced and dried or fried peanut product, the oil is also used in cooking. Peanut ball (Kulikuli), which is a derived product from peanut, is an important traditional food snack and appetizer which is widely consumed in Nigeria. It contributes to overall dietary protein intake for the large segment of the population. Peanut ball is a popular food item with long history of consumption especially in the diet of the low-resource classes of the population in West Africa (Fagbemi,*et al*., 2006; Oshodi and Aletor1993; Aletor*et al*., 2007; Alteschul and Wilcks, 1985).

Mycotoxins are toxins produced by fungi under special conditions of moisture and temperature. Mycotoxins are secondary metabolites of moulds which are of public health significance (Varga *et al*., 2005). Mycotoxin contamination of foods compromises their safety, so their prevention and control are of utmost importance to farmers so as to reduce loss in sales, for exporters to reduce consignment rejection and for the teeming population to reduce food shortage (Aroyeun and Adegoke, 2007). The adverse effects of mycotoxins in man include genotoxicity, carcinogenicity, mutagenicity, teragenocity and immunotoxicity (IARC, 1993). Symptoms of mycotoxicosis range from skin irritation to birth defects, neurotoxicity and death (ICMSF, 1996). Mycotoxins can also cause liver, kidney or other organ damage as well as being cancerous (Forsyth, 1999).Mycotoxins that affect not only food production but also food manufacturing includes patulin, ochratoxin, zearalenone, fumonisins, trichothecenes and aflatoxins (Patricia *et al*., 2006).

Aflatoxin may contaminate many foods including peanuts, corn, cottonseed, apples, milk, oats, barley, silage, wheat, potatoes. Much of the aflatoxin in peanuts is the result of wounding of pods by insects and subsequent invasion by aflatoxigenic fungi present in soil (Dorner, 2003).Aflatoxin contamination of peanuts and peanut products such as peanut ball has been reported to be the highest compared with other food groups like vegetable oil and fat, cereals and cereal product (Lund *et al*., 2000).These mycotoxins occur in several

chemical forms designated aflatoxin B1, B2, G1 and G2 as the four primary aflatoxins plus two additional metabolic products M1and M2 (Dorner, 2004).The "B" and "G" designations refer to the blue or green fluorescence observed upon exposure of the toxin to ultraviolet radiation, M1 is the predominant metabolite of aflatoxin B1 in milk from lactating humans and animals that consume aflatoxin B1 contaminated food or feed. (Patricia *et.al.* 2006). Also, M2 is hydroxylated metabolite of aflatoxins B2 and Aflatoxin B1 is the most toxic and usually predominant form (FAO and WHO, 1997).

Aflatoxins are chemically stable in foods and are resistant to degradation under normal cooking procedures and it is difficult to eliminate aflatoxin once it is produced (Pitt, 2000). Accumulation of aflatoxin is dependent upon weather condition before harvest and the risk for the development of aflatoxin is greatest during major droughts. During post-harvest stage, proliferation of aflatoxin can be accelerated in susceptible commodity under storage conditions of hot and humid storage environment of Africa and some part of China (Pitt, 2000).

Aflatoxins has been reported to be stable in foods and resistant to degradation under normal cooking procedures and this necessitated recommendations from Joint Expert Committee on Food Additives of FAO (JECFA) that the amount present in crops and foods should be reduced to the lowest levels (Herrman*et al*., 2001). Aflatoxin has been reported as probably the worst common mycotoxin by the Australian Mycotoxin newsletter, 1999. Human exposure to aflatoxin is through ingestion of contaminated foods as aflatoxin is not only a potent hepatotoxin, it is also highly carcinogenic (Joe *et al*., 2003). Aflatoxin has also been linked to mental retardation and lowered intelligence (Caster *et al*., 1986). Symptoms may include fever, vomiting and jaundice and acute liver damage may be fatal in severe cases, however, acute toxicity of aflatoxins in human is rare (Peraica *et al*, 1999).

This finding has necessitated the use of *Aframomum danielli*, which has been reported to possess antimicrobial effect on food spoilage microorganisms (Adegoke and Skura, 1994). *Aframomum danielli* has been used as inhibitor of *A. ochraceus* known to be responsible for the production of ochratoxin A (OTA) (Adegoke and Skura, 1994). With the reports in literature of the inhibitory effects of*A. danielli*on aflatoxin-producing moulds (Adegoke and Skura, 1994) and with no information on the use of *A. danielli* in peanut balls, a product that has been libeled as having the highest contamination of aflatoxin. Therefore, this work was planned with the following objectives; to determine the anti-aflatoxigenic effect of *Aframomum danielli* on peanut balls (kulikuli) and to determine the acceptability and sensory preference of peanut balls treated with *A. danielli*.

II. Materials And Methods

2.1. Raw Materials and Preparation

Raw peanuts were purchased from Bodija Market in Ibadan, Oyo State, Nigeria. The anti-aflatoxigenic agent, *Aframomum danielli* pods were obtained from Bode Market, also in Ibadan. Prior to utilization, the seeds were freed of all extraneous materials, pulverized using blender and packaged in airtight polythene bag.

2.1.1. Peanut Ball Production

Ordinary roasting of the peanut was done with the production of both skinned and deskinned peanut ball using different quantities of *Aframomum danielli* (0.1*g*, 0.05*g* and 0.025*g*). One hundred grams of raw peanut was used for each treatment. It was sun dried for four hours before being roasted in the oven(Crown Star Oven toaster (MC 2020 SOT)at 100ºC for 50 minutes with intermittent stirring to obtain even roasting. After roasting, the raw material was spread on a tray to allow for cooling. The samples to be used for kulikuli without the skin was deskinned by rubbing between the palms of hands to remove the skin and then winnowed. It was milled using kitchen blender (Kenwood) in order to avoid contamination from commercial hammer mill. 100g of the fine paste was transferred into mixing bowls and to each one hundred grams sample was added 0.1*g* 0.05*g* and 0.025*g* (equivalent to 100ppm, 50ppm 25ppm respectively*)* of *Aframomum danielli*, 2g of powdered dry pepper (*Capsicum spp*.), 5g of onion slices and 2g of salt were added as additive. The mixture was thoroughly kneaded as it requires vigorous kneading for the oil to be extracted out of it. After the oil extraction, the cake became hard and sticky. It was then molded into different shapes before being fried in its own oil, the samples were then coded.

2.2. Determination of Proximate Analysis

Proximate analysis of the samples was carried out in trilicate according to the official method of analysis described by Association of Official Analytical Chemist (AOAC, 1990).

2.3. Determination of Total Aflatoxin Using Enzyme Linked Immunosorbent Assay procedure (ELISA)

Each Sample was comminuted in a sampling mill to powdered form and 5g sub sample from each sample was weighed into weighing bottles and analyzed using standard methods (AOAC 1990). Sample extraction was done

by adding 25ml of distilled water. The mixture was vigorously shaken for three minutes on a horizontal shaker. In order to get the filtrate, the mixture was filtered into collecting tube using Whatman No 4 filter papers.

2.4. Determination of Total Aflatoxin

Enzyme- Linked Immunosorbent Assay (ELISA), Agraquant method (Rome Labs, Italy) was used for quantifying aflatoxins in test samples. Briefly, all reagents for the ELISA were equilibrated to room temperature before use. 200µL of the assay diluent was transferred into each mixing well. 100µL of the standards or samples were pipetted into the appropriate wells and a multi-channel pipette was used to mix the liquids in the wells 3 times. 100µL of the mixture was transferred to the appropriate antibody-coated wells in duplicate and incubated at ambient temperature for 15 minutes. The wells were washed three times with wash solution and tapped dry. 100µL of the enzyme substrate was added to each microwell and the plate was incubated at ambient temperature for 5 minutes. 100µL of stop solution was added to each well. 100µL of red stop solution was then transferred to each well to stop the reaction before reading the absorbance using a micro well reader within 2 minutes after the addition of the red colour stop solution at 450nm using a differential filter of 630nm

III. Sensory Evaluation

The organoleptic assessment of the treated peanut ball samples (*kulikuli*) was carried out.The panelists were selected by screening on the basis of having consumed and being familiar with the product. Scoring system: 4– much better than control, 3–better than control; 2-same as control; 1- worse than control was used.

IV. Statistical Analysis

All analysis was conducted in triplicate and the mean data of the score was recorded. Data were subjected to analysis of variance and Duncan Multiple range comparison test were performed to separate the means.

V. Result And Discussion

5.1. Proximate Composition

Results of chemical analysis are shown in table 1. Raw peanuts had crude protein value of20% as indicated in table 1 which is closer to that of 25-32% reported by Putnam *et al*., (1991); and a value of 23-25% recorded for peanut ball was close to the 32.4% found by Aletor and Ojelabi, (2007). Sample $PbWS_0$ had crude protein value of 24.50 ± 0.1%; Sample $PbWS_1$ processed with the skin containing 0.025g (50 *ppm*) of the spice had crude protein value of 25.30± 0.2%; Sample $PbWS_2$ also processed with the skin containing 0.05g (50*ppm*) of *Aframomum danielli* had a 25.60± 0.2%. $PbWS_3$ treated with 0.1g (100ppm) of the *Aframomum danielli* had 25.67±0.1% protein. Also, for samples processed without the skin from $PbWiS_0$ to $PbWiS_3$ the protein value ranges between 23.40 ±0.1 and 24.93±0.1. These results indicate that there is increment in protein when peanut ball was produced with the skin of the peanut than when the skin was removed. Also, as the treatment increases so does the protein, fat and crude fiber increases. Carbohydrate and moisture content reduces as the treatment increases both for the product treated with the skin and without the skin(Table 1).

Table 1: Proximate composition of Peanut balls treated with *Aframomum danielli*[a]

Sample code	%CP	%FAT	%CF	%ASH	%MC	%DM	%CHO
RP	.90±0.1	52.60±0.2	3.20±0.2	7.80±0.1	9.25±0.3	98.75±0.2	10.50±0.2
$PbWiS_0$	23.40 ±0.1	44.80±0.1	1.40±0.1	3.40±0.1	1.50±0.1	90.50±0.2	27.00±0.1
$PbWiS_1$	24.75±0.2	45.40±0.1	1.53±0.1	3.80±0.1	1.40±0.2	98.60±0.1	24.52±0.2
$PbWiS_2$	24.83±0.2	46.85±0.1	1.69±0.1	3.80±0.1	1.25±0.1	98.75±0.1	22.85±0.1
$PbWiS_3$	24.93±0.1	48.51±0.1	1.60±0.1	2.80±0.1	1.30±0.1	97.80±0.1	20.96±0.1
$PbWS_0$	24.50±0.1	50.40±0.1	1.70±0.2	3.30±0.2	2.20±0.1	98.70±0.1	18.80±0.2
$PbWS_1$	25.30±0.1	50.50±0.1	2.80±0.1	3.70±0.1	0.60±0.1	99.40±0.1	17.70±0.1
$PbWS_2$	25.60±0.1	51.60±0.1	2.86±0.1	5.50±0.2	0.45±0.1	99.55±0.1	14.44±0.1
$PbWS_3$	25.67±0.1	61.80±0.1	2.90±0.1	2.60±0.1	1.00±0.1	99.00±0.2	17.03±0.1
SD	2.01	2.89	6.18	1.62	3.73	4.23	5.13
CV%	8.07	5.93	294.30	45.00	2.27	4.33	25.15

Level of aflatoxin found in raw peanuts; untreated peanut balls and treated peanut balls are shown in Table 2. The RP had the highest quantity of 5.8µg/kg of aflatoxin. The total aflatoxin content of shelled Peanuts of 5.8µg/kg compares favourably with the 20µg/kg (sum of B_1, B_2, G_1, G_2) action level as the maximum residue limit allowed in food for human consumption except for milk (FAO, 1996) for the purpose of overall sanitary precaution, enacted by the European Union in 1998. This happens as a result of strict adherence to Good Manufacturing Practice (GMP) on the raw material used. However, it was still high considering the severe aflatoxin tolerance standard of 4µg/kg total aflatoxin for nuts and cereals for human consumption (CEC, 1989). Moisture is an important parameter that enhances mould growth and subsequently encourages production of toxic metabolite like aflatoxin (Goldblattt, 1970). The raw peanut used in this study had a slightly higher

moisture content of 7-8%, than the optimum moisture content level of 6-7 % (BCCA, 1996) for stored dry peanuts. Akano and Atanda (1990) found aflatoxin B_1 concentrations in the range of 20-455 µg/kg in peanut balls (kulikuli) purchased from markets in Ibadan, Oyo State, Nigeria. Adebajo and Idowu (1994) reported that most of the corn-groundnut snack contained aflatoxins above 30µg/kg immediately after preparation. Also, aflatoxin B1 concentration of between 25.54 and 455.22 µg/kg as also been detected from peanut product (kulikuli) sold in markets in Benin (Euloge *et al*, 2012). Depending on the processing method of roasting used the total aflatoxin content of the raw material was reduced from 5.8µg/kg to 2.4 µg/kg which is equivalent to 58.6%. This indicates that application of heat during roasting of the nut has effect on the initial aflatoxin load on the raw material. This is in accordance with the findings of Ogunsanwo *et al*., 2004 who observe that roasting have positive effects on reduction of aflatoxin content in peanut seeds.

Samples $PbWiS_1$ PbWiSand$PbWiS_3$ with *Aframum danielli* powder treatment at levels of 25, 50 and 100 ppm and aflatoxin values of 0.7, 1.8 and 2.1µg/kg respectively, representing 87.90%, 69.07% and 63.80% reduction in aflatoxin compared with raw peanut used for the experiment. However, when these aflatoxin figures are compared with processed but not treated sample $PbWiS_0$of2.4 µg\kgit corresponds to 70.8%, 25.0% and 12.5% reduction. This result has shown an inverse relationship to the quantity of the antiaflatoxigenic agent (*Aframum danielli*) used.(Table 2)

Table 2: Effects of *Aframomum danielli* on Total Aflatoxin Reduction of Peanut balls Processed without the skin and with skin[a].

Sample code	**Treatment** (ppm)	**Aflatoxin** (µg\kg)	**Percentage Reduction From Raw**	**Percentage Reduction From Raw**
RP	0	5.8	0	0
$PbWiS_0$	0	2.4	58.6	0
$PbWiS_1$	25	0.7	87.9	70.8
$PbWiS_2$	50	1.8	69.0	25.0
$PbWiS_3$	100	2.1	63.8	12.5
$PbWS_0$	0	3.3	43.1	0
$PbWS_1$	25	0.9	84.5	72.7
$PbWS_2$	50	1.1	81.0	66.6
$PbWS_3$	100	1.5	74.1	54.6

PbWiS: Peanut ball Without Skin, PbWS :, Peanut ball With Skin, Subscript 0,1,2 &3: Treatment 0,25,50 & 100 ppm respectively, Superscript a : mean of triplicate samples. RP: Raw Peanut.

Samples $PbWS_0$, $PbWS_1$, $PbWS_2$ and $PbWS_3$ were processed with skin, sample $PbWS_0$ which was prepared without the spice i.e. at 0 spice levels had aflatoxin content of 3.3µg/kg. (43.1% reduction) when compared with the unprocessed (raw peanut) sample. Samples $PbWS_1$, $PbWS_2$ and $PbWS_3$ were treated with 25, 50 and 100 ppm of the spice had aflatoxin content of 0.9, 1.1 and 1.5µg/kg respectively, representing 84.5, 81.0 and 74.0% aflatoxin reduction from raw peanut seeds respectively (Table 2). To know the effect of the different quantity of *Aframumum danielli*when it is compared with the processed but 0ppmof the spice, $PbWS_0$ contains 3.3µg/kg with values of 0.9, 1.1 and 1.5µg/kg for the three treatments levels corresponding to 72.7%, 66.6% and 54.6% respectively. It was observed that higher aflatoxin reductions were achieved when the peanut balls were produced with the skin of the seeds intact than when the skin was removed. This is represented in Table 2. The inhibition of *A. danielli* powder on Ochratoxin A causing organisms has been reported (Adegoke and Skura, 1994) and other workers, Basilco *et al*., (1990) have reported the inhibition of mycotoxin producing organisms using essential oil of *A. danielli* on other food products but not on peanut balls.

Also, control of ochratoxin A to zero level in*kunun zaki* by incorporating *Aframomumdanielli* was also reported by Adegoke *et al.,* (2007). With ochratoxin, also, Aroyeun and Adegoke (2007) found reduction in mochratoxin A at higher concentrations of the essential oils and extracts than at lower concentration of *A. danielli* for spiked cocoa powder and beverages. However, the minimum levels of aflatoxin of 0.7 and 0.9 µg/kg were obtained at 25*ppm* of the powdered spice; this indicates that the lower the concentration of *A danielli* used, the more the reduction efficiency, that is, the lower the quantity of aflatoxin in the sample.The percentage coefficient of variation in aflatoxin distribution between the samples processed without skin and those processed with the skin was calculated to be 61.8% and 84.16% respectively. This indicates higher quantity of aflatoxin in samples produced with the skin of nuts intact than those produced without the skin.

The mean sensory scores for peanut balls samples treated with varying quantities of *Aframomum danielli* powder are shown in Table 3. The result revealed significant difference($p>0.05$) in the sensorial quality variables for all the samples except for flavor and crunchiness which were rated low for samples $PbWS_0$ and$PbWS_2$. Sample$PbWiS_3$ was generally rated higher for all the attributes and overall acceptability, which has qualified peanut ball produced without the skin and treated with 100ppm of the anti-aflatoxigenic agent.

Table 3: Mean Organoleptic scores of colour, taste, flavour and crunchiness for Peanut balls treated with *Aframomum danielli* powder.

Sample Code	Colour[c]	Taste	Flavour[d]	Crunchiness[e]	Overall Acceptability
$PbWiS_1$	3.80^a	2.10^a	2.00^a	1.80^b	2.45
$PbWiS_2$	3.87^a	2.10^a	1.93^b	1.93^b	2.47
$PbWiS_3$	4.00^a	2.70^a	2.60^a	3.53^a	3.21
$PbWS_0$	1.60^b	1.60^a	1.60^b	1.47^b	1.57
$PbWS_1$	1.83^b	2.00^a	1.73^b	1.60^b	1.79
$PbWS_2$	1.93^b	2.00^a	1.87^b	1.13^b	1.73
PbWS	3.67^a	2.70^a	2.70^b	3.00^b	3.08

Mean with the same superscript along the same column are not significantly different at 5% Level.
c- Includes appeal; d- includes aroma; e –includes ease of fragment.

VI. Conclusion

Peanut cake products are good sources of proteins. However, judging from the results obtained by Akano and Atanda (1990) and Euloge *et al*,. 2012 of Aflatoxin B1 concentration of 20-455.22 µg/kg from peanut ball (*kulikuli*) purchased from markets in Ibadan, Nigeria and Benin, Benin Republic which is of great public health concern. The use of *Aframomum danielli* powder has proved as an effective anti-aflatoxigenic agent for peanut balls and other peanut product. The peanut ball samples shows increasing reduction efficiency with decreasing quantity of the *A.danielli* powder used which conclude that the lower the concentration of the spice used, the more effective it is against aflatoxin. It is concluded that further decrease in the *Aframomum danielli* than 25*ppm* level would result in total elimination of the total aflatoxin in the peanut ball.

Sample $PbWiS_3$ which was treated with 100ppm of *Aframomum danielli* powder had the most positive organoleptic effect as judged by the panelists, particularly with reference to taste, aroma and crunchiness The addition of *Aframomum danielli* powder show remarkable influence on the taste, aroma and crunchiness at higher levels than at lower levels of addition. *Aframomum danielli* powder can be used as anti-aflatoxigenic agent to reduce aflatoxin to lowest minimum level as a food safety measure to minimize the risk of aflatoxin exposure, improve economic sustainability and reducing all potential hazards associated with aflatoxin ingestion by human.This study has also confirmed the reports of Ashaye *et al*. (2006), Aroyeun and Adegoke (2007) that *Aframomum danielli* can be used in food processing and preservation.

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