Orji *et al*
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Evaluation of Aflatoxin contents of Maize, Sorghum and akamu Samples of cereal-based pap enriched with soybean in Abakaliki, Ebonyi State, Nigeria.

Orji J.O., Ayogu T.E., Inyogu J.C., Elom E.E. and Aleke K.T.
Department of Microbiology Ebonyi State University Abakaliki.

ABSTRACT

Aflatoxin contents of Maize, Sorghum and akamu Samples of cereal-based pap enriched with soybean in Abakaliki, Ebonyi State, Nigeria was investigated. Varieties of maize, sorghum and soybean seeds randomly purchased from the international market were subjected to steeping at ambient temperature. Soybean seeds were prepared by washing, soaking, dehulling and parboiling at 100 ^{0}C for 20 min before being wet-milled to slurry. Ground samples of cereal and soybean were mixed together in different ratios and fermented to yield the enriched akamu. The enriched and unenriched akamu (traditionally processed) as control were analyzed for aflatoxin contents using standard chemical and Thin-layer Chromatography method. Aflatoxin detection and identification showed that the aflatoxin levels reduced from 13.3 μgkg^{-1} in white maize to 0.21 μgkg^{-1} in white maize akamu, from 11.13 μgkg^{-1} in yellow maize to 1.25 μgkg^{-1} in white maize akamu, from 6.1 μgkg^{-1} in white sorghum to 2.03 μgkg^{-1} in white sorghum akamu, and from 7.12 μgkg^{-1} in red sorghum to 1.24 μgkg^{-1} in red sorghum akamu. In conclusion, fermented blends of cereal grains and soybean slurry showed drastic reduction on aflatoxin content of akamu samples compared to their primary grain samples.

Keywords: Aflatoxin, Maize, Sorghum, akamu and soybeans.

INTRODUCTION

One of the popular indigenous cereal-based fermented foods in Nigeria is *akamu* or *ogi*, a kind of pap, which is a fermented cereal porridge made from maize (*Zea mays*), sorghum (*Sorghum vulgare*) or millet (*Pennisetum typoideum*) [1,2,3,4]. Ogi can be simply described as a kind of diet that does not require chewing. The cereal-based ogi is very smooth in texture and has a sour taste reminiscent of that of yoghurt. Typically, Ogi has a distinct aroma and fine texture [5]. The colour of the Ogi is mainly depending on the type of feedstock used for the processing. It could either be consumed as porridge (pap) or as a gel-like product (*agidi*) in some West African countries [6]. It is an essential weaning food for infants as well as a dietary staple for adults in West Africa [7,8,9,10]. Generally, traditional cereal foods play a vital role in the diet of the people of tropical Africa particularly in cereal producing regions [11,12]. Ogi production, despite many reported research works is still on a small scale. In this production, domestic equipment are often used [13,14]. Soaking and sedimentation have been widely reported as two important fermentation stages involved in the traditional method of processing ogi [15]. Soaking is often carried out at room temperature [16]. The soaking period is expected to reduce the hardness of the maize grain while the sedimentation period gives the required tartness often desired by some consumers [17,18].The soaked grains are then washed, wet milled, sieved and allowed to sediment for 24–48 h. The ogi slurry may then be processed into varieties of products for infants, children and adult's meal [19].

Aim of the Study

This study is aimed at evaluation of Aflatoxin contents of Maize, Sorghum and akamu samples of cereal-based pap enriched with soybean in Abakaliki, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study Area

The study area of this research was Abakaliki Metropolis of Ebonyi State, Nigeria. Ebonyi State is located in the south-eastern part of Nigeria which lies approximately within longitude 7 ^{0}30 ^{1} and 7 ^{0}E and, latitude 5 ^{0}40 ^{1} and 6 ^{0}45 ^{1} N. It has a population of 149,683, and a land mass of about 5,935 square kilometers. Ebonyi State is bounded to the north by Benue State, to the south by Abia State and to the east by Enugu State and west by Cross River State respectively. Abakaliki, the State capital, has tropical climate with an average relative humidity of 75 % and may reach 80 % during rainy season.The vegetation characteristics are predominantly rainforest with atmospheric temperature of 30 ^{0}C. Two seasons are distinguishable in Ebonyi State: a dry season (November to March) and a wet season (April and October). Abakaliki resident utilizes various cereal-based food products for weaning purposes and general nutritional purposes.

Sample Collection

Exactly 2 kg each of white and yellow maize, white and red sorghum grains and 1 kg of soybean seed were randomly bought from traders at the Abakaliki international market Ebonyi State, Nigeria (i.e. the 5 samples were bought from 5 different shops). The samples were collected in polythene bags from the market and transported to the Applied Microbiology Laboratory of Ebonyi State University, Abakaliki for analysis. The cereal grains were authenticated by a taxonomist in the Department of Crop science, Faculty of Agriculture and Resource Management as *Zea mays, Sorghum bicolor* and *Glycine max* respectively.

Preparation of Cereal-Based Akamu

Preparation of ogi was done according to the methods of [4]. 500g of each cereal grains were washed in distilled water to remove dust particles. The washed grains were steep in 1 Litre autoclaved distilled water for 48 h in covered cleaned plastic containers. Water was decanted and the fermenting grains were re-washed twice to reduce fermenting odour and then wet-milled with a blender. Wet milling of the different cereals grains was followed by wet-sieving to remove bran, hulls and germs. The waste was restrained on the muslin cloth and later dried as poultry feed, while the filtrate was fermented for another 24 h to yield ogi which is either white, cream or chocolate coloured depending on the pigment of the cerealgrain.

Aflatoxin Extraction, Detection and Quantification Extraction

The aflatoxin extraction, detection and quantification was done in the Department of botany Federal University of Agriculture, Makurdi, Benus State.Exactly 1 g of sample was pipetted into a 100 ml conical flask. 2.5 ml of distilled and 25 ml of Chloroform was added. The flask was covered with a stopper and shaked in a shaker for 30 min after which the solution obtained was filtered using a Whatman no.1 filter paper. 10 ml of each extract or filtrate was collected and evaporated to dryness to a volume of 5 ml on a hot water bath at 40 ^{0}C. Five ml extract was stored in dark bottles in a freezer for detection and quantification [18].

Detection of aflatoxin

1 ml chloroform and 0.2 ml of the reconstituted extract was spotted on a pre-coated 20 × 20 cm TLC plate along with aflatoxin standards of

known concentration. The spotted TLC plate was developed in an equilibrated tank containing chloroform: acetone (9:1 v/v). The developed TLC plate was air-dried at ambient temperature (28 ± 2° C) and aflatoxins were detected under UV light at a wavelength of 360 nm. A colour change from blue to yellow upon exposure to aqueous sulphuric acid (50:50 v/v) confirmed the presence of Aflatoxin B1. Aflatoxin B2 was derived from Aflatoxin B1 as dihydro derivative which experienced a colour change from pale blue to deep yellowish colour upon exposure to aqueous sulphuric acid (50:50) to confirm its presence. Aflatoxin G1 fluoresced yellowish green upon exposure to UV light while Aflatoxin G2 fluoresced pale yellowish green upon exposure to same UV light.

Quantification of aflatoxin: 0.5 µm thick preparative TLC plates was employed for the quantification of stored extract after aflatoxin extraction was applied to the plate as a band rather than a spot to chromatograph, the maximum amount of sample at the same time. The preparative TLC plates were developed in an equilibrated tank as an aflatoxin extraction. When the solvent front had risen to about ¾ of the total length of the plate, the plate was taken out of the tank and examined under UV light. The area containing the toxin of interest was located and scrapped off, elute with chloroform and filtered using Muslin cloth. The extract was evaporated to dryness over a hot water bath and reconstituted with 3 ml chloroform. The 3 ml reconstituted solution and aflatoxin standard of 20 µg/ml concentration was used to read Absorbance or Optical Density on an ultraviolet Spectrophotometer (Cecil Instrument CE505) at a wavelength of 360nm[16].

Statistical Analysis

The raw data obtained in the course of the study was presented as mean ± standard deviation in tables and bar charts while relevant data was interpreted using simple descriptive statistics such as minimum, maximum, and one way analysis of variance (ANOVA) with the aid of IBM Statistical Package for Social Sciences (SPSS) version 22 and Microsoft Excel 2013 software. $P < 0.05$ was considered to be statistically significant.

RESULTS

Aflatoxin Analysis of Maize, Sorghum and akamu Samples

There was a drastic decrease in the total aflatoxin levels in akamu samples compared to their primary cereal samples. The aflatoxin levels reduced from 13.3 μgkg^{-1} in white maize to 0.21 μgkg^{-1} in white maize akamu, from 11.13 μgkg^{-1} in yellow maize to 1.25 μgkg^{-1} in white maize akamu, from 6.1 μgkg^{-1} in white sorghum to 2.03 μgkg^{-1}in white sorghum akamu, and from 7.12 μgkg^{-1} in red sorghum to 1.24 μgkg^{-1} in red sorghum akamu. There was a significant difference ($P < 0.05$) between Aflatoxin B_1, B_2, G_1 and G_2. Total aflatoxin in yellow maize (11.13 μgkg^{-1}) was significantly ($P < 0.05$) higher than that of white maize (9.31μgkg^{-1})(Table1)

Table 1: Aflatoxin analysis of Maize, Sorghum and akamu Samples

Sample	Sample Code	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total Aflatoxins	P-value
Maize (μgkg^{-1})	WM	3.11 ± 0.08	3.0 ± 1.01	2.1 ± 1.17	1.1 ± 1.69	9.31	0.012
	YM	3.7 ± 1.65	3.8 ± 1.88	2.6 ± 1.66	1.03 ± 1.55	11.13	0.01
Sorghum (μgkg^{-1})	WS	3.1 ± 0.02	2.0 ± 0.03	0.99 ± 0.07	0.01 ± 0.55	6.10	0.008
	RS	3.6 ± 0.55	2.2 ± 0.13	1.3 ± 0.38	0.02 ± 0.53	7.12	0.014
Akamu(μgkg^{-1})	WM_O	0.08 ± 1.05	0.07 ± 0.91	0.05 ± 0.85	0.01 ± 0.55	0.21	0.094
	YM_O	1.12 ± 0.68	0.09 ± 0.90	0.03 ± 0.87	0.01 ± 0.55	1.25	0.129
	WS_O	1.02 ± 0.72	0.9 ± 0.50	0.1 ± 0.80	0.01 ± 0.55	2.03	0.054
	RS_O	0.65 ± 0.85	0.42 ± 0.74	0.15 ± 0.76	0.02 ± 0.53	1.24	0.002

KEY: WM_5 = White maize at 500g (unenriched), YM_5 = Yellow maize at 500g, WS_5 = White sorghum at 500g, RS_5 = Red sorghum at 500g, WM_4 = White maize at 400g (enriched), YM_4 = Yellow maize at 400g, WS_4 = White sorghum at 400g, RS_4 = Red sorghum at 400g, WM_3 = White maize at 300g (enriched), YM_3 = Yellow maize at 300g, WS_3 = White sorghum at 300g, RS_3 = Red sorghum at 300g, WM_2 = White maize at 240g (enriched), YM_2 = Yellow maize at 250g, WS_2 = White sorghum at 250g, RS_2 = Red sorghum at 250g. SD = Standard Deviation.Values represent means of data ± Standard Deviation (SD). Data was statistically analyzed at 95% level of confidence ($P < 0.05$).Standards Organization of Nigeria. Standard for Aflatoxin composition of Cereals = 4 μgkg^{-1}

DISCUSSION

Aflatoxins and Sensory organoleptic quality of the unenriched and enriched cereal-based akamu

The detection of aflatoxin however in fresh ogi although in minute quantity is the residual aflatoxin content of the samples that were not totally removed from the sample during the processing. Aflatoxins are heat-stable metabolites that are able to persist in food products even after the aflatoxigenic fungus that produced them had died. [3], reported that the preformed aflatoxin in the aflatoxin contaminated maize persisted till the end of processing maize into akamu slurry. However, the quantity of the aflatoxin in the fresh ogi obtained from fresh and stored maize poses no threat to the safety of the product [9]. Maize is a cereal that has been severally reported to be susceptible to aflatoxins contaminations. The contamination of stored and fresh maize samples with aflatoxin is consistent with some reports. [12], stated that certain environmental factors stimulate the extent to which mycotoxins are produced, which varies with geographic location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing periods. Aflatoxins contamination of food products poses a serious threat to human's and animal's health, while its toxicity has remained a topic of debate in the international market as well as economic development of the country which are part of trade market [9]. Many researchers have widely reported high levels of aflatoxin contamination of maize and maize products in Nigeria. Maize as an important agricultural commodity throughout the world is considered as one of the best substrate for the growth of fungi and produce toxicogenesis [8].The drastic decrease in the total aflatoxin levels in akamu samples compared to their primary cereal samples whereby aflatoxin levels reduced from 13.3 μgkg^{-1} in yellow maize to 0.21 μgkg^{-1} in white maize akamu, from 11.13 μgkg^{-1} in yellow maize to 1.25 μgkg^{-1} in white maize akamu, from 6.1 μgkg^{-1} in white sorghum to 2.03 μgkg^{-1}in white sorghum akamu, and from 7.12 μgkg^{-1} in red sorghum to 1.24 μgkg^{-1} in red sorghum akamu. This study affirms the findings of [11] who reported that new maize and old maize obtained from a local market in Kaduna had a mean aflatoxin level of 102 ppb and 177 ppb respectively. The result of the higher level of aflatoxin recorded in maize (11.13µg/kg-1) compared to sorghum (7.12 µg/kg-1) can be attributed to the storage time of both cereal crops coupled with other factors such as temperature, insect damage, and other environmental factors [15]. During storage of this grains, it has been discovered that aflatoxigenic fungi are able to grow and release aflatoxins into various food products [7].

The result of the aflatoxin contamination levels in both maize and sorghum samples is far below the acceptable aflatoxin level of 4 ppm for maize set by Standard Organization of Nigeria (SON) [6, 9, 10] thereby making them safe for consumption. However, due to the fact that aflatoxins accumulate in grains during storage, if the maize and sorghum samples used for this study had spent more time in storage they could have accumulated a higher concentration of aflatoxins which could be above the acceptable level thereby making them unsafe for consumption [11]. According to [16], high level of aflatoxin in food products is unacceptable and possible legal action to eliminate such products from the market may be taken by the government. The reduction in the aflatoxin level of both maize and sorghum-based akamu compared to the raw cereal samples that were used to process them could be as a result of various processing methods that were used during their production. This finding is in agreement with the work done by [11] who stated that traditional methods of processing maize products are effective in

reducing Aflatoxin B1 content in the final products. Findings from a similar study carried out by [13] agreed to the fact that processing methods applied during the processing of maize into various food products reduced the aflatoxin in maize. The first processing method that the maize samples were subjected to was fermentation. Fermentation of maize has been proven to be effective in reducing aflatoxin in cereal products [6]. [11], reported that the fermentation of maize samples during the processing of 'Doklu' (a fermented maize product consumed in Cote d'lvoire), caused a significant reduction in the concentration of total aflatoxins (72%) of the maize samples with most aflatoxin B1 (80%) after the soaking of maize grains for 72 hours. Also, [13] had reported that aflatoxins levels in maize grains significantly ($p < 0.05$) reduced by 50% (from 50 µg kg-1 to25µgkg–1) after 72 hours of fermentation. Another factor that could be responsible for the reduction in the aflatoxin content in the akamusamples of both maize and sorghum akamuis the ability of the Lactic acid bacteria such as *Lactobacillus plantarum* to detoxify the aflatoxin content of the maize during fermentation. According to the report of [13], among all the lactic acid bacteria tested for the reduction of aflatoxin in maize samples that were artificially contaminated with aflatoxin, *Lactobacillusplantarum*, the major lactic acid bacterium that participates in the fermentation of maize into akamu was discovered to be the most effective as the highest rate of reduction of aflatoxin was recorded in the maize samples inoculated with the strains of the bacterium.

The second stage of the production of the akamu samples which could have led to the reduction of aflatoxin content in the maize samples was sieving of bran from the fermented maize/sorghum slurry.[3] had reported that aflatoxin in maize grains concentrate in the bran. The report of [5] is in agreement with this finding as they found out that after fermenting maize grains that contained aflatoxin level of 50 µg/kg and sieving its slurry to form ogi, the aflatoxin level in the akamu sample was found to be 25 µg/kg while the remaining aflatoxin content had adsorbed unto the bran. This is also affirmed by Sule*et al.* [9] who collected maize grains, maize flour and maize bran from a market in Kaduna state. They reported that among all the samples collected maize bran sample had the highest level of aflatoxin with a mean aflatoxin level of 213 ppb. In a similar report by [8], the dehulling of maize grains samples made the aflatoxins levels to significantly decrease from 10.7 and 270 ng/g with a mean value of 87.3 $\mu gg^{-1}$to 6.8 $\mu gg^{-1}$and 182 μgg^{-1} with a mean value of 57.3 μgg^{-1}. They also reported that the aflatoxin contents in the by-products, comprising hulls and fines, were 2-7 times higher than the levels in the whole-grain maize and ranged from 103 μgg^{-1} to 613 μgg^{-1}.

Enriched white and yellow maize in this study were the most preferred by 100 % of 10 judges in the survey among the enriched cereal-based akamu presented. Meanwhile, unenriched red sorghum akamu was the least choice, with only 69 % of the judges appraising it. Based on the study of [5] on the organoleptic characteristics of ogi supplemented with spice (ginger and clove), colour, appearance, texture, taste and overall acceptability were preferred for sieved ogi with spice while the flavor of sieved ogi without spice were preferred. Similarly, [9] reported that akamu supplemented with 10% moringa leave showed apparent difference (less level of sensory acceptability) with plain ogi with regard to taste, mouth feel and flavor but showed no significant difference. The authors showed that colour, appeal and general acceptability of 100% maize ogi is preferred showing significant variation. However, in all cases of sensory quality, its range from slight likeness to very much likeness. [10] reported that blended with mango

mesocarp and soybean has significant acceptability with regard to texture, colour, flavor and general acceptability compared to unblended akamu. [5] also reported that akamu without okra seed meal or roasted okra seed meal had superior organoleptic properties (aroma, colour, taste, texture and general acceptability). [11], reported that 5% ginger blend to cooked ogi improved the appearance, taste, texture colour and aroma.

CONCLUSION

There was a drastic decrease in the total aflatoxin levels in ogi samples compared to their primary cereal samples, whereby aflatoxin levels reduced from 13.3 μgkg^{-1} in white maize to 0.21 μgkg^{-1} in white maize ogi, from 17.13 μgkg^{-1} in yellow maize to 1.25 μgkg^{-1} in white maize ogi, from 6.1 μgkg^{-1} in white sorghum to 2.03 μgkg^{-1} in white sorghum akamu, and from 8.12 μgkg^{-1} in red sorghum to 1.24 μgkg^{-1} in red sorghum akamu.

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