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MYCOFLORA AND AFLATOXIN CONTAMINATION OF KOKORO-A NIGERIAN MAIZA SNACK

Onifade D.A.[1] --- Adesokan I.A.[2] --- Adebayo-Tayo B.C.[3]

[1]*Department of Science Laboratory Technology, The Polytechnic, Ibadan, Nigeria*

[2]*Department of Science Laboratory Technology, The Polytechnic, Ibadan, Nigeria, Dept. of Microbiology, CSK Himachal Pradesh Agricultural University, Palampur, India*

[3]*Department of Microbiology, University of Ibadan, Nigeria*

ABSTRACT

Kokoro is maize snack which is very popular among poor masses in Nigeria who consume it along with gari(a cassava product) as lunch on regular basis. In this study fungal contaminants of kokoro were characterized and its aflatoxin content determined. A total of 30 fungal isolates were obtained from kokoro samples and they belong to 3 different species. Aspergillus flavus had the highest frequency of occurrence of 73.33% while Penicillium species had the lowest (6.66%). Different concentration of aflatoxin B_1 was detected in some of the kokoro samples analyzed. Sample D had the highest concentration of 7.25 parts per billion (ppb). The lowest concentration detected was 0.06 ppb in sample P. No aflatoxin G_1 and G_2 was detected in all the kokoro samples with exception of sample P which contained 2.54 ppb aflatoxin G_1.According to international standards some of the kokoro samples are not suitable for human consumption because of high level aflatoxin which was above the recommended level. Therefore, production of kokoro should be standardized and appropriate packaging materials utilized to prevent the growth of aflatoxigenic fungi. This is to safeguard the health of many poor Nigerians who consume it on a regular basis.

Keywords: Kokoro, Maize snack, Aflatoxin, Contamination, Mould, Nigeria.

1. INTRODUCTION

There is always the need and desire to eat in man, but its adequacy really matters. This adequacy is determined by the quality and effective utilization of the nutrient consumed. In most part of Nigeria, people depend on ready made convenient foods for their nutritional requirement.

Such food include biscuit, bread, cakes, roasted corn, fried maize paste (*kokoro*) and others. These foods can serve as main meals or in between snacks for both children and adults.

The saga of human nutrition and the improvement of human health has been reflected in the effort of many scientists who have belief that human performance and well being both physical and mental depends primarily on what is eaten [1].

Living at the present is dangerous not only because of accidents and environmental hazards, but also because of the foodstuffs that we consume every day. Most foods are contaminated with microorganisms as well as their toxins [2]. Mycotoxins are some of the most potent toxic compounds known to man [3]. Aflatoxins are found in many countries of the world, especially in tropical and subtropical regions where the warm humid weather provides optimal conditions for the growth of aflatoxinogenic moulds. The optimum temperature for the growth of moulds is 24 – 35°C and equilibrium relative humidity of above 70%. Thus food and feed grown under tropical and subtropical conditions are more prone to aflatoxin contamination than those in temperate regions. Nigeria, being a tropical country, its conditions of temperature and humidity are favourable for growth of the moulds and production of the toxin [4]. The prevalence of human exposure to aflatoxin has been shown to be over 98% in West Africa, including Nigeria. In the World Bank report mycotoxin-induced diseases led to a reduced life expectancy in developing countries [5].

Intake of these aflatoxin contaminated foods above the level considered to be safe may be harmful to human beings and other animals [6]. The potential of aflatoxin as a carcinogen, mutagen, tetratogen, and immunosuppressive agent is well documented [7]. The aflatoxin contamination of many food commodities have been reported [6, 8, 9]. However, aflatoxin contamination of *kokoro* is scarcely reported. Therefore, the objective of this present study is to identify fungal contaminants and determine the level of aflatoxin contamination in *kokoro* samples obtained from local producers.

1.1. Materials and Methods

1.1.1. Collection of Samples

Sixteen samples of maize snack (*kokoro*) were purchased from street hawkers and local markets in Ibadan metropolis and transported to the laboratory in sterile containers for immediate analysis.

1.1.2. Isolation and Identification of Fungi

Fungal isolation was carried out by the method described by Jonathan and Olowolafe [10]. One gram of each sample was grinded and separately diluted serially in sterile distilled water. 0.1 ml of each dilution was seeded into Petridishes containing sterile potato dextrose agar (PDA) in which 0.05mg of streptomycin sulphate has been added to suppress the growth of bacteria. The plates were incubated for 7 days and the fungi developed were purified by repeated streaking on PDA. Identification of the fungi was carried out according to the description of Alexopoulus, et al. [11].

1.1.3. Determination of Aflatoxin

The aflatoxin analysis was carried out using a combination of AgraQuant kit. Using a shaker at room temperature, 10g of each kokoro sample was extracted with 20ml methanol: water (70:30). The residue was dissolved in 1 ml of methanol: water (3:1,v/v) and 200ml of diluted extract was applied to the enzyme immunosorbent assay (ELISA) plate (Romer Lab. AgraQuant) in order to determine the total aflatoxin content. Each one of both samples and standards were applied in duplicate. The ELISA was performed according to the manufacturer's instructions. The intensity of the resulting yellow colour was measured in the ELISA plate reader with an absorbance filter of 450nm and evaluated according to the RIDAWIN program. The optical densities (ODs) were then compared to those of the standards. Aflatoxin concentration in each sample was expressed as parts per billion (ppb) (12).

1.2. Analysis of Data

All the experiments were carried out in triplicates and data generated were subjected to analysis of variance (ANOVA). The tests of significant were carried out using Duncan's multiple range test (DMRT).

2. RESULTS AND DISCUSSION

A total of 30 different fungal isolates were obtained from samples of *kokoro* and they belong to 3 different genera (Table 1). *Aspergillusflavus*had the highest frequency of occurrence of 73.33% while *Penicillium* spp. was the lowest 6.66%. The presence of these fungal species might not be unconnected with crude method of production, lack of proper packaging materials and poor storage condition. The high percentage species occurrence of *A. flavus* should call for concern since it has been implicated in the production of different type of aflatoxins [4]. Maize and maize products provide an excellent substrate for mould growth and mycotoxin contamination [8].

Different concentration of various group of aflatoxins were detected in *kokoro* (Table 2). The highest concentration (7.25ppb) of aflatoxin B_1 was detected in Sample D and the lowest concentration (0.65ppb) of aflatoxin B_2 detected was in sample P. Aflatoxins G_1 was not detected in all the samples with the exception of sample P which contained 2.54 ppb. However, aflatoxin G_2 was not detected in all samples of *kokoro* analyzed. For overall sanitary precaution, the European Union has enacted very severe aflatoxin tolerance standards of 2ppb aflatoxin B_1 and 4ppb total afflatoxins in dry food product for human consumption [5]. Therefore, by international standards, some of the *kokoro* samples analyzed are not fit for human consumption.

Unfortunately, most poor Nigerian masses consume *kokoro* with gari as their afternoon meal on regular basis. In the light of this the production of *kokoro* should be standardized; and appropriate packaging materials used to eliminate or reduced drastically the risk of aflatoxin contamination to safe guard the health of Nigerian populace that consume *kokoro* on regular basis [12].

Table-1. Fungal contaminants of *kokoro* and their frequency of occurrence (%).

Fungal isolates	Occurrence	Frequency of occurrence (%)
Aspergillus flavus	22*	73.33
Aspergillus niger	6	20.00
Penicillium spp.	2	6.66
Total	30	100

*Values are means of three different determinations.

Table-2. Aflatoxin concentration (ppb) in samples of kokoro analysed.

S/N	Sample code	B_1	B_2	G_1	G_2	B-toxins	G-toxins
1	A	0.00*	0.00	0.00	0.00	0.00	0.00
2	B	0.00	0.00	0.00	0.00	0.00	0.00
3	C	0.75	0.00	0.00	0.00	0.75	0.00
4	D	7.25	0.00	0.00	0.00	7.25	0.00
5	E	0.00	0.00	0.00	0.00	0.00	0.00
6	F	4.29	0.77	0.00	0.00	5.07	0.00
7	G	0.00	0.00	0.00	0.00	0.00	0.00
8	H	1.09	1.04	0.00	0.00	2.13	0.00
9	I	0.78	0.83	0.00	0.00	1.61	0.00
10	J	0.93	0.60	0.00	0.00	1.53	0.00
11	K	0.00	0.65	0.65	0.00	0.65	2.54
12	L	0.00	0.00	0.00	0.00	0.00	0.00
13	M	5.11	1.57	0.00	0.00	6.68	0.00
14	N	1.16	0.00	0.00	0.00	1.16	0.00
15	O	0.00	0.00	0.00	0.00	0.00	0.00
16	P	0.00	0.00	0.00	0.00	0.00	0.00

*Values are means of three different determinations.

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