Toxigenic fungal contamination of cocoa-based beverages: A possible public health concern in a tropical country

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Abstract

Food safety is a call for concern nowadays. Food borne disease and microbial spoilage of food result from the failure of or inability to control microorganisms at one or more stages of food chain, from raw material production to consumption of the final product. This study was undertaken to screen some cocoa-based beverages sold in Nigeria in order to ascertain the mycological and aflatoxin status of such foods. Seventy-nine (79) samples of different brand of cocoa beverages collected from different markets in Benin City, Nigeria was evaluated by estimating the fungal load; using standard plate count method, and aflatoxin B1(AFB1) level by immunoaffinity silica gel column extraction and thin layer chromatography with spectrophotometric detection. Colonies of mould isolated from the samples were identified by standard mycological methods as Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus. Zamis beverage sample recorded the highest fungal count of 5500 cfu/g, AFB1 level of 40.6 ± 3.2 µg/kg and moisture content of 4,00%; while Peak beverage sample recorded the least fungal count of 500 cfu/g, AFB1 level of 5.3 ± 2.5 µg/kg and 1.00% moisture content. AFB1 was not detected in Ovaltine and Benco beverage samples. The most frequent genera of moulds in all samples was A. flavus, having an incidence of 63.3%. Sachet cocoa-based beverages sold in Benin metropolis carry potential health hazard. Thus, improved handling through food processing, preservation and storage can minimize aflatoxins in foodstuffs and ensure sustainable quality of food supply. This findings suggest needs for urgent attention to the possible public health implications.

Keywords: Aflatoxin B1; cocoa beverages; moulds; Public health.

Contaminación fúngica toxigénica de las bebidas a base de cacao: una posible preocupación de salud pública en un país tropical

Resumen

Las enfermedades transmitidas por los alimentos y su deterioro microbiano son el resultado de la incapacidad de regular o controlar los microorganismos en una o más etapas de la cadena alimentaria, desde la producción de la materia prima hasta el consumo del producto final. Este estudio se realizó para detectar algunas bebidas a base de cacao que se venden en Nigeria, con el fin de determinar el estado micológico y aflatoxínico de dichos alimentos. Setenta y nueve (79) muestras de diferentes marcas de bebidas de cacao recogidas de diferentes mercados en la ciudad de Benín (Nigeria), se evaluaron mediante la estimación de la carga de hongos; utilizando el método de recuento de placa estándar y el nivel de aflatoxina B1 (AFB1) por extracción de columna de gel de sílice de inmunoafinidad y cromatografía de capa fina con detección espectrofotométrica. Las colonias de moho aisladas de las muestras se identificaron mediante métodos micológicos estándar como Aspergillus flavus, Aspergillus niger y Aspergillus fumigatus. La muestra de bebidas Zamis registró el mayor recuento de hongos de 5500 ufc /g, nivel de AFB1 de 40,6 ± 3,2 µg/kg y contenido de humedad de 4,00%; mientras que la muestra de bebidas Peak registró el menor recuento de hongos de 500 ufc /g, el nivel de AFB1 de 5,3 ± 2,5 µg/kg y el contenido de humedad del 1.00%. AFB1 no se detectó en muestras de bebidas de Ovaltine y Benco. Los géneros más frecuentes de moho en todas las muestras fue A. flavus, con una incidencia de 63,3%. Las bebidas con bolsita de cacao que se venden en la metrópolis de Benin conllevan un riesgo potencial para la salud. Por lo tanto, una mejor manipulación a través del procesamiento, la conservación y el almacenamiento de los alimentos puede minimizar las aflatoxinas en los alimentos y garantizar una calidad sostenible del suministro de alimentos. Estos hallazgos sugieren la necesidad de una atención urgente a las posibles implicaciones para la salud pública.

Palabras Clave: Aflatoxina B1; bebida cacao; mohos; salud pública.

INTRODUCTION

In recent years food and feed safety has been a major concern of nations especially as more knowledge is gathered on the occurrence of natural toxins in food stuffs, fertilizers, animal feed and edible plant materials. World Health Organization (WHO) has characterized naturally occurring toxins as significant sources of food borne illnesses (1), while the Food and Agriculture Organization (FAO) has estimated that fungal toxins alone contaminate about 25% of agricultural products worldwide resulting in great losses for farmers (2) (3).

Food safety is usually determined by the absence or presence of pathogenic organisms, or their toxins, and the number of pathogens, with their expected or destructive agents (4). The level of spoilage microbes reflects the microbial quality, wholesomeness, of a food product as well as the effectiveness of measures used to control or destroy such microbes (5). Food borne disease and microbial spoilage of food result from the failure of or inability to control microorganisms at one or more stages of food chain, from raw material production to consumption of the final product (6). Specifically, the microbiological tools are used to assess the safety of food, adherence to good manufacturing practices (GMPs), the keeping quality (shelf life) of certain perishable foods and the utility (suitability) of a food or ingredient for a particular purpose (7).

In Nigeria, like other tropical and sub-tropical regions of the world, aflatoxicosis is a public health problem and control of aflatoxin contamination requires thorough risk assessment, monitoring, quality control and empirical data (8). Aflatoxin problem is global; however, it is more serious in tropical countries of the world where relative humidity is high and temperatures conducive for the growth and production of aflatoxin by moulds. Aflatoxin are potent carcinogens that are produced as secondary metabolites of strains of Aspergillus parasiticus and Aspergillus flavus that grow on important food crops such as groundnuts, maize, cocoa and other oilseeds (9).

The consumption of cocoa-based beverages is fast gaining ground in Nigeria due to its nutritional and health benefits. Its production has been an increasing trend in Nigeria without much concern for whether or not they meet the microbiological criteria for food safety and public health consequences (10). Cocoa powder has a reduced water activity that may not constitute suitable substrate for the growth of microbes, but if not handled in hygienic form before consumption can result in the production of pathogenic organisms or production of toxic metabolites that can cause serious health problems (10).

Moulds are frequently found in cocoa beans and it is not uncommon to find mycotoxin-producing moulds and occasionally low levels of mycotoxins in cocoa (11). Beside Aspergillus being among the fungus genera, it has also been implicated in mycotoxicosis because it produces toxic metabolite called mycotoxins in food. Some of the species of this genus that have been severally reported in mycotoxicosis includes Aspergillus flavus, which produces aflatoxin that causes cancer of the liver, Aspergillus ochraceous and Aspergillus niger which produce ochratoxin that is nephrotoxic (4).

In view of this, there is need to determine the mycological safety of the cocoa based beverages we consume as health drink so as to stem down the occurrences of mycotoxin associated diseases in our community. Also, regardless of the wide consumption of these group of food by Nigerians, little or no data are available as regards mycotoxin levels in the commodities; the need for this study.

In this research, we screened some sachet cocoa beverages retailed in Benin metropolis, Edo State, Nigeria for aflatoxin B1 levels and fungal load with the aim of providing preliminary useful data on the aflatoxin status of these foods consumed in many homes and to enlighten the manufacturers and consumers on the need for proper food processing, handling and storage.

MATERIALS AND METHODS

Collection of samples

Seventy-nine (79) samples of different brands of the beverages (Zamis, Domo, Milo, Cowbell, Richoco, Ovaltine, Bournvita, Spectra, Benco and Peak chocolate) were obtained from four different markets (Uselu Market, New-Benin Market, Oba Market and Zoro supermarkets) in Benin City (Nigeria). The samples were obtained at two-week intervals for 24 weeks to obtain a good representation. Samples were analysed mycologically within 24 h of collection.

Evaluation of mycoflora

The evaluation of fungi was carried out using dilution plating method and the direct plating technique (12). Decimal dilutions of the samples were carried out by placing one gram (1.0 g) of the beverage powder into 9.0 ml of sterile distilled water. This was thoroughly shaken and from the suspension, 1.0 ml was transferred to another tube containing 9.0 ml of sterile distilled water and thoroughly mixed again. This dilution procedure was further repeated thrice so that there were series of five tubes giving a serial dilution of 10-1 to 10-5. An aliquot of 1.0 ml was pipetted at each dilution into sterile Petri dishes. Three of the plates were over-laid with cooled molten potato dextrose agar (PDA). The remaining three were over-laid with Harold agar. The latter contained malt, 40 % sucrose and yeast extract which makes it suitable for isolating osmophilic and xerophilic moulds.

Each medium was supplemented with 0.60 µg/ml of streptomycin sulphate to suppress bacterial growth. The plates containing the beverage powder and melted agar were swirled round to allow for thorough mixing of aliquot and media. After the agar had gelled, the plates were incubated at room temperature (28±2°C) for 5 to 7 days. The number of fungal colonies that appeared in a plate was multiplied by the dilution factor to obtain the number of colony forming units per gram (cfu/g) of cocoa-based beverage. For direct plating on agar media, 1.0 g of each sample was aseptically plated on PDA and Harold agar. The plates were incubated under room conditions (28±2°C) and examined after 7 days under a stereoscopic binocular microscope for the presence of fungi.

Representative colonies of fungi that appeared on agar plates were repeatedly sub-cultured on fresh PDA until pure culture of each isolate was established. Identification of fungi was by observing the growth habits and morphological characteristics under a wild binocular microscope. Wet mounts of hyphal/asexual structures stained with lactophenol in cotton blue were viewed under the compound microscope and identified with reference to standard texts (13) (14). Characterization of the fungi was done based on the colour of the colony, appearance, conidiophore, mycelium, arrangement of conidia on sterigmata. The pure culture of fungi got was prepared on a clean glass slide and stained with cotton blue in lactophenol. Observation was done under ×40 oil immersion objective lens.

Estimation of total fungal counts

The total fungal in the samples was estimated from the decimal dilutions carried out. The incidence of mould contamination using direct inoculation was expressed as a percentage of the 79 samples examined.

Determination of pH and moisture content

The pH of each sample was analysed using the pH meter. Moisture content of each sample was determined using Automated Moisture Analyzer (Sartorius MA 150, Germany) as described by the (15). The method was based on loss of moisture upon drying at 105oC.

Extraction and determination of aflatoxin

The beverage samples were extracted with chloroform, and the extract was concentrated in vacuum. The dry material was transferred to 1-dram vials with small amounts of chloroform. The solution was evaporated to dryness under a stream of nitrogen. The crude extract was cleaned up by silica gel column (15). Aflatoxins were dissolved in chloroform and separated by thin layer chromatography on silica gel 60 plates using chloroform-methanol (97:3 v/v) as the developing solvent. The spots of aflatoxin B1 were removed from the plates, eluted with methanol and estimated spectrophotometrically with absorbance read at 365nm (16).

Statistical Analysis

The data obtained were subjected to analyses using the one-way analysis of variance (ANOVA) in SPSS statistical package and statistical significance was accepted at 5% probability level or less.

RESULTS AND DISCUSSION

The total fungal counts and moisture content in the samples varied from 0.5 x 103 to 5.5 x 103 cfu/g and 1.00 to 4.00 respectively with an average pH value of 7.15 for all samples (Table 1). Altogether, 3 fungal species belonging to Aspergillus mould (A. flavus, A. niger, A. fumigatus) were identified with A. flavus mainly isolated; having an incidence rate of 63.3%. Aflatoxin B1 levels was not detected in 2 samples (Ovaltine and Benco) but identified and quantified in 8 of the samples analysed; with amount varying from 5.3µg/kg to 40.6µg/kg.

Table 1. Total fungal count per gram of beverage and some physicochemical parameters

Beverage sample	Fungal count (103cfu/g)	Moisture content (%)	pH value
Zamis	5.5	4.00	7.02
Domo	5.0	2.00	7.04
Milo	4.5	2.70	7.60
Cowbell	4.5	1.23	7.08
Richoco	4.0	3.02	7.70
Ovaltine	4.0	1.00	7.01
Bournvita	2.5	3.00	6.98
Spectra	2.5	1.03	7.01
Benca	2.0	1.03	7.01
Peak	0.5	1.00	7.08

Table 2 shows the allowable limits (4.0µg/kg) specified by the European Commission (17) which is also currently being used by the National Agency for Food and Drug Administration and Control (NAFDAC), in Nigeria. Zamis sample had the highest fungal count and aflatoxin B1 level which correlated directly (r = 0.91) with its moisture content amongst all sample analysed. It has been demonstrated that most of these food–borne fungi exhibit the potential to produce toxic metabolites.

There is sufficient evidence to conclude that naturally occurring mixtures of aflatoxins are carcinogenic to animals and humans (18). Some mycotoxins are tremorgenic i.e. cause novel neurotoxic effects; muscular tremors in animals.

Beverage sample	Total No of sample	flavus (%)	A.niger (%)		AFB1 (µg/kg) sA.fumigats (%) A.fumigats (%) A.fumigats
Zamis	10	8(10.1)	5(6.3)	7(8.9)	40.6(3.2)
Domo	10	4(5.1)	3(3.8)	-	10.5(4.1)
Milo	10	6(7.6)	3(3.8)	-	15.3(2.2)
Cowbell	9	8(10.1)	4(5.1)	-	24.6(2.0)
Richoco	8	7(8.9)	3(3.8)	6(7.6)	24.6(2.0)
Ovaltine	8	2(2.5)	4(5.1)	4(5.1	-
Bournvita	8	7(8.9)	5(6.3)	4(5.1)	18.2(3.5)
Spectra	6	4(5.1)	-	-	13.2(2.8)
Benca	5	2(2.5)	-	-	-
Peak	5	2(2.5)			5.3(2.5)
Overall total	79	50(63.3)	27(34.2)	21 (26.7)	154.2(22.9)

Tremorgens are produced mainly by species of Aspergillus and Penicillium. Also, they have been known to produce mycotoxins such as aflatoxin, ochratoxins, aflatrem, aspergillic acid and aspertoxin. Mycotoxins seem able to cause serious disease of the liver, kidney and blood – forming organs in extremely low quantities i.e. parts per billion. In addition, many mycotoxins have been shown to impair immunity against various pathogenic agents. This has been demonstrated for aflatoxins, diacetoxyscripenol, ochratoxin and rubratoxin (19) (20).

Again, in humans, mycotoxins have been implicated in a form of encephalopathy observed in Thailand and in a particular nephropathy rather frequently seen in the Balkans (14). Apart from danger of food poisoning caused by these fungi, they also utilize nutrients found in the food thereby causing deterioration of such food. To improve the quality of cocoa beverages and to prevent spoilage at various water activity (aw), it was suggested by Mossel and Shennan (21) that if the aw is below 0.65 and the product is maintained at this level during storage, problems arising due to microbial spoilage are rare, irrespective of the number of contaminating organisms present. It was further noted that aflatoxins production ceases or become very low at aw below 0.85 However, high level of contaminating organisms should be avoided,

since they can survive for long periods and may contaminate other foods or cause problems after dehydration.

Pelczar et al., (22) also reported that the extent of contamination will depend upon the initial microbiological quality of the product and the level of aseptic precaution used during handling. Therefore, if the product is well handled, the level of microbial content of the final product will be minimal. Furthermore, the spectrum of the fungi isolated is similar to those in the raw material. This may be due to reinfection of the product during cooling of the samples before they are packaged into polyethylene bags because of the ubiquity of these organisms.

A more recent and increasingly popular way of preserving foods is the use of controlled storage or modified atmosphere packaging (MAP). These methods take advantage of combining the inhibitory effect of low O2 levels and elevated CO2 levels in any deterioration processes in foods as well as preventing the microbial spoilage (23).

The results of our investigation demonstrate that the aflatoxigenic fungus (Aspergillus) aided by moisture and other factors is a common agent of contamination of cocoa-based beverages marketed in Benin City, Nigeria. However, not all samples contain the B1 aflatoxin screened for; but the levels of Aflatoxin B1 in most of the food samples were generally above the maximum allowable limits of NAFDAC. Reduction of aflatoxin levels in food stuffs in Nigeria especially in cocoa-based beverages should be a public health priority.

Values in parentheses for AFB1 levels are S.D. of three replicates

Conflict of interest

The authors have declared no conflict of interest.

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