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Microbiological Study of Fungal Contaminants in 'Agidi': A Commercially Produced Cereal Food in Akoko Communities, Nigeria

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ABSTRACT

Samples of 'Agidi' food commonly consumed fermented food products in Akokoland, Nigeria were purchased from Obada, Okore and Osele markets of Ikare-Akoko community, Nigeria. This food source was examined for occurrence of aflatoxigenic mould contamination. The results obtained shows that the mould count ranged from 95×10^3 sfu/mL to 12×10^5 sfu/mL; 195×10^3 sfu/mL to 55×10^5 sfu/mL; 82×10^3 sfu/mL to 62×10^5 sfu/mL; 44.33×10^3 sfu/mL to 10×10^5 sfu/mL; 43×10^3 sfu/mL to 37×10^5 sfu/mL; 55×10^3 sfu/mL to 14.33×10^5 sfu/mL; 45×10^3 sfu/mL to 33×10^5 sfu/mL; 21×10^3 sfu/mL to 5×10^5 sfu/mL and 15.33×10^3 sfu/mL to 4×10^5 sfu/mL respectively for the samples examined. Aspergillus spp., Fusarium spp., Saccharomyces spp., Wardomyces spp. were the most prevalent species obtained from Agidi samples identified. Other isolates obtained belong to the fungal group Geotrichum and Fusarium spp. The most prevalent strains isolated and studied further were Aspergillus flavus, Saccharomyces cerevisiae, Fusarium solani and Fusarium oxysporum. The Aspergillus flavus are usually responsible for aflatoxigenic mould contamination. Isolation of mould and aflatoxin producing strains of Aspergillus flavus in this study are of significance because they are sources of public health threat. It is recommended that high level of hygiene be maintained in the preparatory processes and production of this cereal food (Agidi) to enhance health safety.

Keywords

Agidi, Akoko communities, Cereal food, Fungal contaminants, Nigeria.

Introduction

Aflatoxins are toxic metabolites produced by different species of toxigenic fungi, called mycotoxins. Humans can be exposed to aflatoxins by the periodic consumption of contaminated food, contributing to an increase in nutritional deficiencies, immune suppression and hepatocellular carcinoma [1]. Aflatoxins (AFs) can be found in different kind of matrices, such as spices, cereals, oils, fruits, vegetables, milk, meat, etc. Among the 18 different types of aflatoxins identified, the major members are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2) which are produced by Aspergillus flavus and Aspergillus parasiticus. A. flavus and A. fumigatus have also been identified as pathogens for animals and humans [2]. The biosynthesis of aflatoxins, as all secondary metabolites, is strongly dependent on growth conditions such as substrate composition or physical factors such as pH, water activity, temperature or modified atmospheres. Aflatoxin is the most investigated mycotoxin in this crop being reported in nine countries at levels of up to 3,282 µg/kg in Brazil [3].

Aflatoxin can be consumed in basic food commodity such as maize kernels and rice as in the case for Nigeria, or, in its ready-to-eat form, e.g. porridge for those leaving in the rural area of Limpopo in South Africa. In order to approximate the level of exposure of Africans to aflatoxin, let's assume a Nigerian that eats 138kg of cereals annually [4] as a representative of Africa and whose main staples are maize and rice consumed in a ratio of about 3:1.the aflatoxinB1 mean values for indigenous maize and rice destined for human consumption in the continent are approximately 258 and 83µg/kg, respectively.

Several diseases are attributed to aflatoxins as they are ubiquitous in African food commodities [5], it is not surprising that several of such conditions are caused or exacerbated by these toxins. The

diseases related to Aflatoxin B1 are discussed below; As the liver is the main organ of detoxification and the first major organ to be exposed to dietary intake of xenobiotics, it is not surprising to find that several liver conditions have been associated with aflatoxin, particularly in under developed countries, especially in Africa. The fact that many African crops, in particular, the staples such as groundnut and maize, can be routinely contaminated with aflatoxin, leads to an intuitive feeling that many diseases, including those of the liver, can be linked directly or indirectly, to aflatoxin, especially AFB1. As already intimated, this is a dangerous supposition, as many chronic diseases are multi-factorial in nature, and as in any scientific hypothesis, needs strong evidence of support. This is not easily gathered in the rural areas of Africa where infrastructure is poor, health services varying from none to basic clinics and health centres with little time or inclination to gather usefully directed statistics. Even the analysis and quality control of staples is dependent upon external scientific studies, which often are one of investigations that merely provide a snapshot of a true situation. Other diseases that can be linked to this are Hepatitis, Cirrhosis, Hepatocellular Carcinoma (HCC), Kwashiorkor, Reye's syndrome.

When considering interventions, several routes may be taken [6]. The best approach is that of prevention which is always better than cure. One such intervention is that of releasing non-aflatoxigenic strains of Aspergillus flavus into the agricultural environment and such a commercial product called Afla-Guard® is available commercially. This results in suppression of naturally occurring aflatoxigenic strains [7]. Another is the introduction of genetically modified variety of crops, e.g., genetically modified (GM) Bt maize which inhibits insect damage and hence fungal infection [8]. Another preventive measure is feeding of animals with amino acids and vitamins particularly lysine and vitamin C that have protective actions against mycotoxins [9,10]. A more traditional way is the use of fungicides and pesticides, although current preference is not in favour of this. The use of natural predators (cats and dogs) at fields and storage sites to deter rodents, birds and monkeys is a very practicable preventive control strategy for Africa. Post-harvest treatments are a little more difficult due to the persistence of aflatoxin in commodities even after processing. Early harvesting, effective drying (to moisture level of less than 14%), cleaning, removal of damaged produce (sorting), e.g., small and discoloured groundnuts [11]; good storage facilities with controlled humidity [12] and packaging can all contribute to lowering the level of the mycotoxin in the final product. While these remain, the most effective post-harvest control measures for Africa, other alternative but less effective measures include reduction of storage time, use of chemical and botanical preservatives, and detoxification of contaminated produce. Legislation to protect consumers against the hazards of mycotoxins is important to reduce this menace.

Fermentation is one of the oldest, commonest and economical methods of processing and preserving foods [13]. This can also help to preserve against the surge of aflatoxin. Fermented cereals based food products produced in African countries can be classified on the basis of either the raw cereals ingredient used in their preparation or texture of the fermented product Classification on the basis of raw cereals ingredients include: Wheat- based foods e.g bouza, kishk; Rice- based food e.g buza; Maize- based foods e.g ogi (agidi), kenkey; Millet- based food e.g kunuzaki; Sorghum-based foods e.g pito, bogobe, kisra, burukutu, injera.

Classification based on food texture include; Liquid (gruel) e.g ogi, mahewu, burukutu, pito, uji. Solid (dough) and dumplings e.g kenkey, agidi and Dry (bread) e.g kisra, injera. Maize is one the principal sources of food in Africa and South America than in the developed world. Cereal consumption was estimated to be more than 100kg per year inhabitant. Recently, an increase of the consumption of maize has been noticed due to an increased population. The introduction of foreign ` high- tech' processing concepts and food products like wheat bread, wheat-based o milk-based weaning foods, yoghurt and larger beer to tropical countries was followed by a rapidly increasing demand during the early post-independence period and of these expensive products provided status [14].

Preparation of Agidi

Clean and healthy maize brought from the market and about 250g was weighed with the weigh balance. They were washed thoroughly with clean water and washed grains was submerged in water and allowed to ferment for three days. The fermented grains with clean sorghum leaves (oroporo) was added then wet-milled with clean water and allowed to ferment for another 2hours. The slurry was cooked without sieving at 1000C for 10-15 minutes by stirring continuously while on fire. The partially cooked slurry was then molded like a ball and wrapped in a sterile cocoa leaves, it was recooked for about 15minutes in a pressure pot to get 'egidi'.

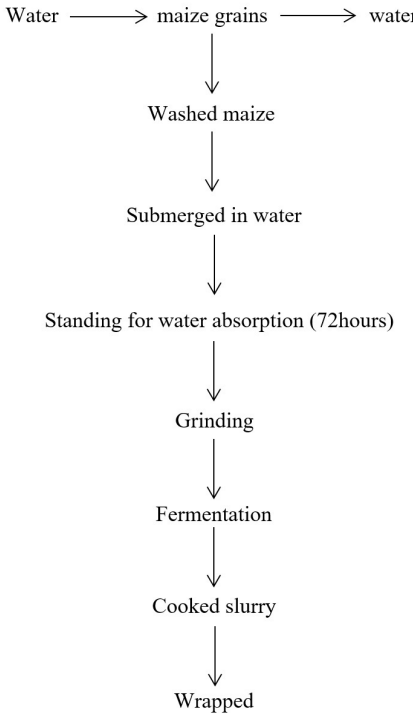

Figure 1: Flow chart of Agidi production.

Agidi is common to the Akoko people of Ondo State, Nigeria.

It can be eaten by dissolving in cold water containing milk in a hot afternoon or eaten with stew or vegetable. The Akoko people fondly call it 'egidi'. According to previous investigators [15-17], the quality and shelf-life of cereals including 'agidi' made from maize can be extended beyond one week by refrigerating at a low temperature with sodium benzoate treatment. This can help to improve the shelf life of 'agidi' in order to maintain its usefulness and increase its popularity as weaning food for infants and as food for adults. It is important to know the occurrence of aflatoxigenic moulds present in 'agidi' sold to consumers to ascertain whether it is safe for consumption after a particular period of time. The main aim of this study is to know the occurrence of aflatoxigenic moulds in Agidi and identify the aflatoxin producing mold(s) from these sources.

Materials and Methods

Sample Collection

The Samples of the fermented cereal product, 'Agidi' were purchased from different markets, namely; Osele, Obada and Okore, all in Ikare – Akoko, Ondo State. Care was taken in ensuring that the samples bought were freshly prepared that very day. The samples were aseptically collected in aclean polythene bag and transported to the Laboratory (Microbiology Laboratory, AAUA), where they were to be worked on. The samples used were both sweet and sour. Equipments used for this study were properly sterilized.

Media Preparation

The media used were Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA) respectively. 35.5g of MEA was weighed using a spatula, a piece of foil paper and a weighing balance. The media was poured inside a conical flask and was then dissolved in 1000ml of sterile distilled water. 65g of SDA was also weighed and dissolved likewise in another conical flask. The mixture was homogenized by gently shaken it and the top of the flask were corked with minutes that the media cotton wool wrapped in a foil paper. This is to prevent the media from spilling off during autoclaving. The media were, autoclaved at a temperature of 121°C for 15 minutes. The autoclaved was then switched off and the steam was allowed to escape making sure that it cooled to 0°C before unpacking the few had been cooled, it was then carefully aseptically dispensed into the sterilized Petri-dishes. The media preparation was done according to the manufacturer's specification.

Serial Dilution

1g of the freshly prepared Agidi sample (sweet and sour) was dissolved in a test tube containing 9ml of sterile distilled water, the dilution was thoroughly shaken to obtain a uniform suspension of microorganisms and diluted serially.

Isolation and Enumeration of Microorganisms

The isolation of microorganisms was done using pour plate method. 1ml each of diluent 10^{-3} and 10^{-5} were dropped into four sterile Petri dishes already arranged on the swabbed workbench. About 20ml of MEA was poured into two Petri dishes and was gently swirled for even distribution of microorganism. 20ml of SDA was also poured into the other two Petri dishes and was swirled. The plates were left to solidify and were then incubated at 25°C for 3-5 days. After 72 hours, the growth was visible enough, the number of colonies per plates were counted and recorded. This was expressed as the Colony Forming Unit (CFU). Isolates obtained were identified by proper microbiological methods.

Identification of pure cultures

A drop of Lactophenol-in-cotton blue was placed on a clean grease free slide, an inoculating needle in either hand was used to remove a piece of mycelium free from the fungal pure culture and placed on the stained slide, teasing it out by means of two pointed needles. A cover slip was carefully placed on it to avoid air bubbles, after which it was examined first under low power(x10) and then under high power (x40) objective lens of a light microscope. The structures seen was drawn and compared with the fungal structures found in a compendium.

Results

This study shows different types of fungal species isolated from 'agidi' a fermented cereal. Twenty four fungal species were isolated from the agidi samples obtained for this study. This includes, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Aspergillus versicolor, Fusarium oxysporum, Fusarium solani, Saccaromyces cerevisiae, Sporothrix skenchii, Geotrichum candidum* and *Wardomyces simplex species* (Table 1).

Table 1 shows the morphological characteristics of fungal isolates for identification purposes. Similarly in Table 2, the relationship between the occurrence and percentage of fungal isolates from samples was determined. Here, *Aspergillus* was the most prevalent 37.51% followed by *Saccharomyces cerevisiae* 29.17%. Other isolated moulds were *Fusarium spp*, *Wardomyces spp*, *Sporothrix spp* and *Geotrichum spp* (Table 2). The cultural characteristics some of the isolates are shown in plates 1-4 for *Aspergillus flavus*, *Aspergillus parasiticus, Aspergillus niger* and *Saccharomyces cerevisiae* respectively. In Table 3, the mean values of total mould counts in examined agidi samples range from the lowest of 15.33×10^3 sfu/mL, to the highest, 195×10^3 sfu/mL, and lowest of 4×10^5 sfu/mL to the highest, 62×10^5 sfu/mL.

Plate 1: *Aspergillus niger*


Plate 2: *Aspergillus parasiticus*

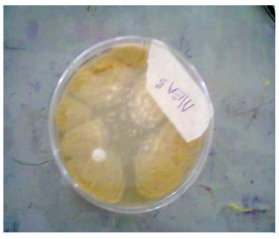
Plate 3: *Aspergillus flavus*


Plate 4: *Sacharamyces cerevisiae*

Table 1: Morphological Characteristics of Fungal Isolates.

Sample	Shape	Appearance	Elevation	Surface	Hyphae	Septa	Spore	Features	Probable organism
M1	Circular	Yellowish green	Flat	Smooth	Branched	Septate	Large conidia	Club shaped microconidia	*Aspergillus flavus*
M1	Circular	Bluish green	Raised	Smooth	Branched	Septate	Large conidia	Conidia are round, single celled and clustered at the end of each conidiophore.	*Aspergillus fumigatus*
M2	Circular	Black	Raised	Smooth	Unbranched	Septate	Conidia found in large conidiophore	Philiade produce chain of mostly round but sometimes rough conidia	*Aspergillus niger*
M3	Circular	Greyish green	Raised	Roughened	Branched	Septate	Conidia with prominently echinulate walls.	Conidiophores are coarsely roughened and metulae absent.	*Aspergillus parasiticus*
M3	Spoon shaped	Greyish-yellow	Raised	Smooth	Branched	Septate	Conidia are very small about 22.5µm	Stipes are relatively short, with heavy yellow walls, vesicles are usually ellipsoidal rather than spherical and bear metulae and phialides only over two third.	*Aspergillus versicolor*
M2	Oblong	White or peach	Raised	Smooth	Branched	Septate			*Fusarium oxysporum*
M3	Canoe	Cream	Flat	Smooth	Branched	Septate	Macroconidia are produced from phialides on branched or unbranched conidiophores	Phialides are cylindrical, with a small collarette, solitary or produced as component of a complex system.	*Fusarium solani*
M2	Box	White to cream	Raised	Smooth	Branched	Septate	They produce arthroconidia which remain cylindrical or ellipsoidal.	Chains of hyaline smooth, one celled. Slimy arthroconidia by the fragmentation of undifferentiated hyphae.	*Geotrichum Candidum*
M1	Elongate	White to cream colour	Flat	Smooth	Absent	Non septate	Blastoconidia are unicellular, globose and ellipsoidal to elongate in shape	Large globose to ellipsoidal budding yeast-like cells or blastoconidia	*Saccharomyces cerevisiae*
M3	Oval to cigar shaped	Greyish to dull brown	Erect	Smooth	Branched	Septate	The conidia rapidly dislodge and the conidia look like budding yeast cells	Conidiophores are sympodial appear weakly differentiated from the vegetative hyphae.	*Sporothrix skenchii*
M2	Oval	Dark brown	Erect	Smooth	Branched	Septate	Annellophores 10-15µm long in their cylindrical part, annellated zone to 30µm long.	Conidia regularly 2- celled, ellipsoidal, with a truncate base, hyaline, smooth-walled.	*Wardomyces simplex*

Discussion

Various fungal species that were aflatoxigenic in nature were isolated from this popular food source 'Agidi' being consumed in some Akoko communities in Nigeria (Table 1). Occurrence of aflatoxigenic mould contamination in fermented cereal – 'agidi' sample could pose threat to public health. Generally, moulds lead to great economic losses and constitute a major production of mycotoxins.

A total of 24 moulds strains belonging to 11 species were isolated and identified from examined samples. This study show that *Aspergillus* was the most prevalent 37.51% followed by *Saccharomyces cerevisiae* 29.17%. Other isolated moulds were *Fusarium spp*, *Wardomyces spp*, *Sporothrix spp* and *Geotrichum spp*. (Table 2). *Aspergillus spp* is widely incriminated as contaminants of human foods [18], especially cereals. The presence of Aspergillus spp is not only of economic importance but also represents a real health hazard. They can be of allergic, toxigenic and pathogenic effect through the production of mycotoxins [19].

Table 2: Percentage of occurrence of fungal isolates from agidi samples.

S/N	Isolates	Frequency of occurrence	Percentage of occurrence
1	*Aspergilus flavus*	5	20.83
2	*Aspergillus fumigatus*	1	4.17
3	*Aspergillus niger*	1	4.17
4	*Aspergillus parasiticus*	1	4.17
5	*Aspergillus versicolor*	1	4.17
6	*Fusarium oxysporum*	2	8.34
7	*Fusarium solani*	2	8.34
8	*Geotrichum candidum*	1	4.17
9	*Saccharomyces cerevisiae*	7	29.17
10	*Sporothrix skenchii*	1	4.17
11	*Wardomyces simplex*	2	8.34
Total=24			

Percentage of occurrence = FOC/N×100/1

KEY: FOC=Frequency of Occurrence of Fungal Isolates

N=Total number of Isolates; M1- Obada market; M2-Okore market; M3-Osele market

Isolates 1, 2 and 9 was gotten from the samples purchased from M1
Isolates 3,6,8 and 11 was gotten from the samples purchased from M2
Isolates 4, 5, 7 and 10 was gotten from the samples purchased from M3.

Table 3: Fungal counts of Fermented Cereal 'Agidi'.

Isolates	No of colony forming unit (CFU)			Mean	CFU/ml x10^3 of sample
	Plate 1	Plate 2	Plate 3		
Sample 1	120	75	90	95	95
	12	11	14	12.33	1233
Sample 2	220	175	190	195	195
	50	45	55	55	5500
Sample 3	85	100	60	82	82
	60	80	46	62	6200
Sample 4	50	58	25	44.33	44.33
	80	65	30	58.33	5833
Sample 5	30	62	34	42	42
	14	10	06	10	1000
Sample 6	36	63	30	43	43
	40	32	20	37	3700
Sample 7	70	55	40	55	55
	20	15	08	14.33	1433
Sample 8	60	40	35	45	45
	50	30	18	33	3300
Sample 9	26	23	14	21	21
	06	05	03	5	500
Sample 10	18	16	12	15.33	15.33
	07	02	02	4	400

Key: Number of CFU = mean number of CFU's × dilution factor (ml).

Table 3 shows that the mean values of total mould counts in examined agidi samples. These range from the lowest of 15.33×10^3, to the highest, 195×10^3, and lowest of 4×10^5 to the highest, 62×10^5. From the public health of view, *Aspergillus spp., Fusarium spp*. and related moulds has been reported to cause diseases such as pulmonary aspergillosis, pulmonary allergy, mycotic keratitis and skin infection [20,21]. Isolated fungi like Aspergillus and *Fusarium* have been considered to be a major leading factor in food spoilage, resulting to great economic losses and constituting a public health hazards by producing varieties of mycotoxins causing food poisoning. The predominant isolated aflatoxin causing species was *Aspergillus flavus*, followed by Fusarium.

Food and Drug Administration (FDA) established regulatory working guidelines on the acceptable levels of aflatoxins in human foods set at 20ppb for total aflatoxins [22]. The most effective means to prevent aflatoxigenic mould contamination of 'agidi' is through the application of strict hygienic measures during the processing. This study shows that the occurrence of relatively high levels of moulds and aflatoxigenic fungi in agidi samples analyzed presents high risk to consumers' health. It is therefore suggested that a statutory food regulating body dedicated to protecting public health and consumer interest in the area of food safety and hygiene be established. And those in existence should be strengthened with appropriate resources. Its main function would be to take all reasonable steps to ensure that food products meets the high standards of food safety and food handlers should observe necessary food production regulations and ethics.

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