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Occurrence of aflatoxins and aflatoxin-producing fungi in ofada rice (Oryza sativa–Oryza *glaberrima* blend) sold in Lagos markets: food safety implications

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Ofada rice is an indigenous variety of rice predominantly grown and consumed in Nigeria. Similar to other cereals, it is susceptible to fungal and mycotoxin contamination. This study investigates the occurrence of aflatoxins and aflatoxin-producing fungi in ofada rice and its food safety risks. Three hundred grams (300 g) of ofada rice were purchased from each of four (4) different markets in Lagos: Victoria Island, Ajah, Mushin and Mile 12 markets, using simple random sampling within each market. Samples were pooled into composites per market. One half (150 g) was cooked while the remaining half (150 g) was uncooked. Five (5) grams each were analysed for the presence of aflatoxin using the LC-MS/MS technique. One gram (1 g) each was used for fungal isolation using Sabouraud Dextrose Agar (SDA).Molds isolated were identified as *Aspergillus flavus* (2 different strains), *Aspergillus tritili and Penicillium* sp. SGE33. LC-MS/MS results showed that aflatoxin levels in both cooked and uncooked ofada rice were below reporting limit of <1ppb. Low levels of aflatoxins in ofada rice in this study, irrespective of the presence of toxigenic *Aspergillus* species, indicate that this cereal is generally safe for consumption, as it poses no food safety or public health risks.

Key words: Ofada rice, aflatoxin, food safety, public health, fermentation.

INTRODUCTION

Rice is one of the most important cereals of the world and is staple to over three billion people, constituting over half of the world's population (Priyanthi et al., 2024). It is an essential source of proteins, carbohydrate, dietary fibre, minerals and bioactive compounds. There are two important varieties namely, Oryza sativa (Asian rice) and Oryza glaberrima (African rice). Ofada rice is a blend of the two and it is indigenous and very popular in the Western part of Nigeria. Ofada rice is unpolished, with some or all of the rice bran left on the grain, enhancing its flavor and nutritional value. The husk of Ofada rice contains more fiber, which is beneficial for digestive health (Oguntunde et al., 2018). It is known for its aromatic qualities and swelling when cooked. It is categorized based on the colour of the un-milled seed as either brown Ofada or white Ofada. Brown Ofada rice is highly aromatic, while white Ofada rice is typically non-aromatic. Ofada rice plays a very important role in the diet of many Nigerians where it is consumed in various forms such as boiled, jollof, fried or tuwo shinkafi (local rice paste). It is used as raw material for the production of local beverages such as kunu, pito and cereal gruels.

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Ofada rice is processed traditionally by parboiling method that involves three stages of treatment: soaking, parboiling and drying (Adekoyeni and Adeboye, 2018). The soaking step involves soaking the milled rice in water for several hours or days, allowing natural yeasts and bacteria to ferment the sugars present. The fermented rice is then parboiled and sun-dried. This rice is specially relished because of its characteristics flavor that develops during soaking stage as a result of fermentative activities by some microorganisms. Otegbayo et al. (2012) reported that soaking (fermentation) contributes mainly to the organoleptic, physical and nutritional changes in the rice. It enhances the nutty aroma and flavour of Ofada rice. Storage of rice is done in bamboo-laid ceilings and barns, baskets and sacks. The prevailing high temperature and relative humidity are such that encourage colonization by fungi. However, proper storage practices may allow a shelf life of up to a year (Adekoyeni et al., 2018).

Generally, rice is cultivated in hot and humid climatic conditions and these encourage the proliferation of fungi and subsequent mycotoxin production. In addition, inadequate storage conditions, and weather conditions such as floods and heavy rainfall at the time of harvest aggravate the situation (Adewunmi and Fapohunda, 2019). According to Reddy et al. (2009) most farmers in Africa including Nigeria practice sun-drying, a method which is not sufficient to reduce the moisture to acceptable levels, thus making rice a favourable substrate for fungal growth. Other reports have indicated the detection of various mycotoxins in rice in Nigeria including aflatoxins at concentrations above EU regulatory limits of 4-15µg/kg for total aflatoxins in cereals (Makun et al., 2011), while others reported lower concentrations within acceptable limits (Ayejuyo et al., 2008). Somorin and Bankole (2010) reported the detection of aflatoxin B1 in ofada rice samples sold in Lagos and Ogun states of Nigeria. However, whether they occur at lower concentrations or not, it remains a public health issue because consumption of small doses over a long period will result in chronic effects in consumers (Adewunmi and Fapohunda, 2022).

Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2) are among the identified mycotoxins in rice (Alameri et al., 2023; Ferre, 2016; Majeed et al., 2018) and are produced by *Aspergillus flavus* and *Aspergillus parasiticus*. AFB1 is considered the most potent and has been classified by International Agency for Research on cancer (IARC) as human carcinogen group 1, hence a primary concern for public health. It readily contaminates a wide range of food products, including peanuts, maize, cottonseed, and tree nuts. Other major mycotoxins found in cereals including rice are fumonisins (FB), zearalenone (ZEN), citrinin (CIT), deoxynivalenol (DON), T-2/HT-2, enniatin (ENs) and beauvericin (BEA), and these are produced by toxigenic fungi such as *Aspergillus, Penicillium, Fusarium* and *Alternaria* spp (Ferre, 2016; Majeed et al., 2018). *Fusarium* and *Alternaria* spp are some of the field fungi that infect rice grains pre-harvest while *Penicillium* and *Aspergillus* spp attack grains during storage (Mohapatra et al., 2017).

Consuming rice contaminated with aflatoxins poses several health risks such as liver cancer, growth impairment in children, male infertility, and damage in DNA and genetic mutations (Jannik et al., 2020). The high daily intake of rice, even with lower aflatoxin levels, is a public health concern as rice is highly consumed in Nigeria. However, despite the evidence of contamination, little is known about ofada rice sold in Lagos markets. Lagos, being a metropolitan city, is highly populated. Overcrowding and congestion can lead to inhalation of high amount of dust containing mold spores, and ingestion of contaminated food including ofada rice that might be exposed to mold spores and mycotoxins from the environment. Therefore, this study is aimed at investigating the occurrence of aflatoxin and aflatoxin - producing fungi in ofada rice sold in Lagos markets in Lagos state, Nigeria. This research is justified due to the quest for the consumption of wholesome and contamination-free ofada rice, and it is significant because the demand for locally grown rice has increased in recent years due to government's push for food self-sufficiency.

MATERIALS AND METHODS

Sample collection

Samples of ofada rice were purchased from four (4) major markets in Lagos State. The coordinates of the sampled markets are: Mile 12 (6°31’41” N 3°23’42” E), Mushin (6°31’43” N 3°21’08” E), Victoria Island (Oniru) (6°26’25” N 3°30’26” E), and Ajah (6°28’17” N 3°33’52” E). Using simple random sampling, 300 g of ofada rice was purchased from six (6) different sellers from each market. All samples purchased were pooled together per market to have a general information on the food safety risks per market. They were collected into sterile airtight zip locked bags and transported to microbiology laboratory for microbial analysis. Figure 1 shows the map of Lagos State.

Sample analysis

Three hundred (300) grams of ofada rice sample from the markets were divided into two (2) parts. One part, 150 g of ofada rice sample was cooked with water for 30 min, while the other part was uncooked. The cooked and the uncooked ofada rice from the four (4) markets was ground into powder with a grinder.

Fungal isolation

1g of each of the cooked and uncooked ofada rice samples was weighed into a test tube containing 9 mL of distilled water and used for 10-fold serial dilution. After mixing, 0.1 mL was transferred from each dilution onto already poured and set plates of Saboraud Dextrose Agar (SDA) containing chloramphenicol (500 mg/L) and streptomycin (500 mg/L) in triplicates. With the use of a bent rod,

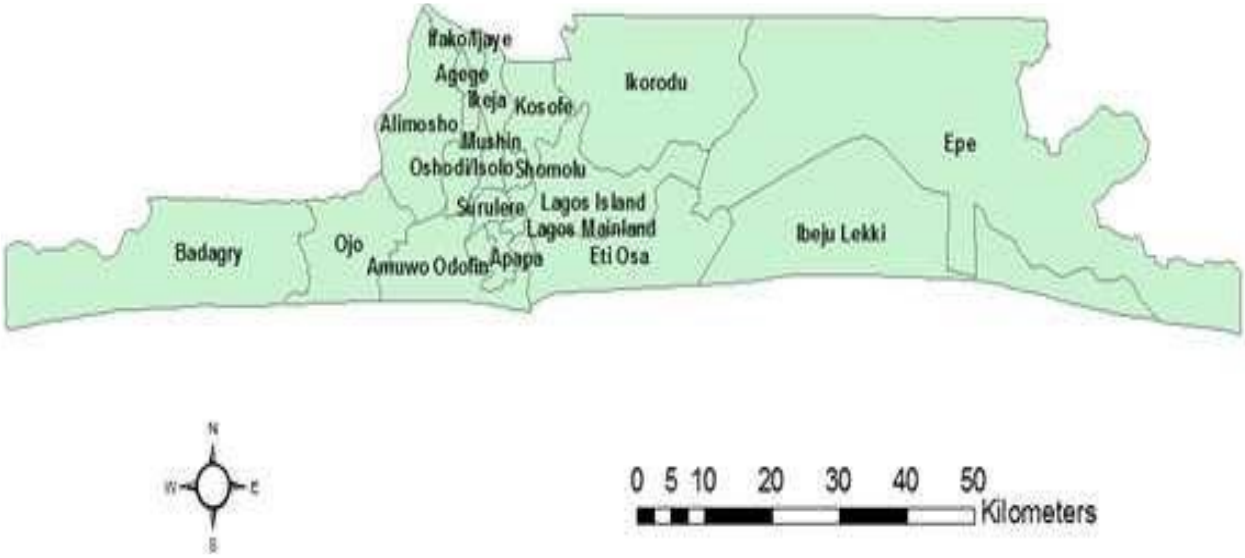

Figure 1. Map of Lagos state.
Source: Olukanni and Oresanya (2018).

the inoculum was spread evenly on top of the agar, using the spread plating technique. It was allowed to dry and incubated at 25°C for 5 days. Fungal colonies from the plates were counted after 5 days and sub-cultured using the 3-point culture technique on fresh SDA plates for purification of the isolates. Pure cultures of the isolate were sub-cultured onto cryovials containing SDA and incubated again for 7 days. These pure cultures were covered with sterile distilled water and kept at 25°C. They were eventually sent for molecular identification in African Biosciences Laboratory, Ibadan, Nigeria.

Aflatoxin analysis of samples

Five grams (5 g) of ground cooked and uncooked rice were sent to Trilogy laboratory Washington, USA for analysis. Aflatoxins associated with the ofada rice samples were analysed using the dilute and shoot LC-MS/MS technique as described by Malachová et al. (2014). Five grams (5 g) of cooked and uncooked ofada rice samples were homogenized with 20 ml of extraction solvent (acetonitrile/water/acetic acid 79:20:1 v/v/v) in a 50 ml polypropylene tube. All samples were extracted for 90 min on a GFL 3017 rotary shaker and diluted with the same volume of the extraction solvent. The diluted extracts were directly injected into the LC-MS/MS instrument. Apparent recoveries of the analytes were determined by spiking 0.25 g of the different samples. The spiked samples were stored overnight at ambient temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and samples.

RESULTS

Fungal load in ofada rice across markets

The total mold counts from each market for both cooked and uncooked ofada rice are shown in Table 1. From the results, the total mold counts for the uncooked rice ranged from 2.6 $x10^2$ cfu/mL in Victoria Island to 3.3$x10^4$ cfu/mL in Mushin Market; while the total mold counts for the cooked rice ranged from 4.0$x10^1$ in Victoria Island to 2.9$x10^3$ in Mushin Market. From the statistical analysis (using ANOVA), fungal counts from Mushin market were significantly different ($p \leq 0.05$) compared to other markets sampled, in both uncooked and cooked ofada rice. However, no significant difference was observed between uncooked and cooked ofada rice ($p = 0.41$).

Morphological and molecular identification of fungal isolates

The morphological identification of isolates from ofada rice is shown in Plates 1 to 4, while molecular identification and DNA sequencing results are shown in Table 2.

Occurrence of aflatoxins in cooked and uncooked ofada rice

The LC-MS/MS results of aflatoxin in both uncooked and cooked ofada rice samples are shown in Tables 3 and 4

DISCUSSION

Market hygiene and storage practices as drivers of fungal contamination

In this study, the total mold counts of uncooked rice from

Plate 1. *Aspergillus flavus* on SDA.

Plate 2. *Aspergillus flavus* Strain ND63 on SDA.

the four (4) markets ranged from 2.6 x 10^2 cfu/mL (Victoria Island) to 3.3 x 10^3 cfu/mL (Mushin)(Table 1). The higher mold counts in Mushin samples likely result from the area's high population density, which contributes to dusty and unhygienic conditions. Poor handling and exposure of Ofada rice to these conditions in markets,

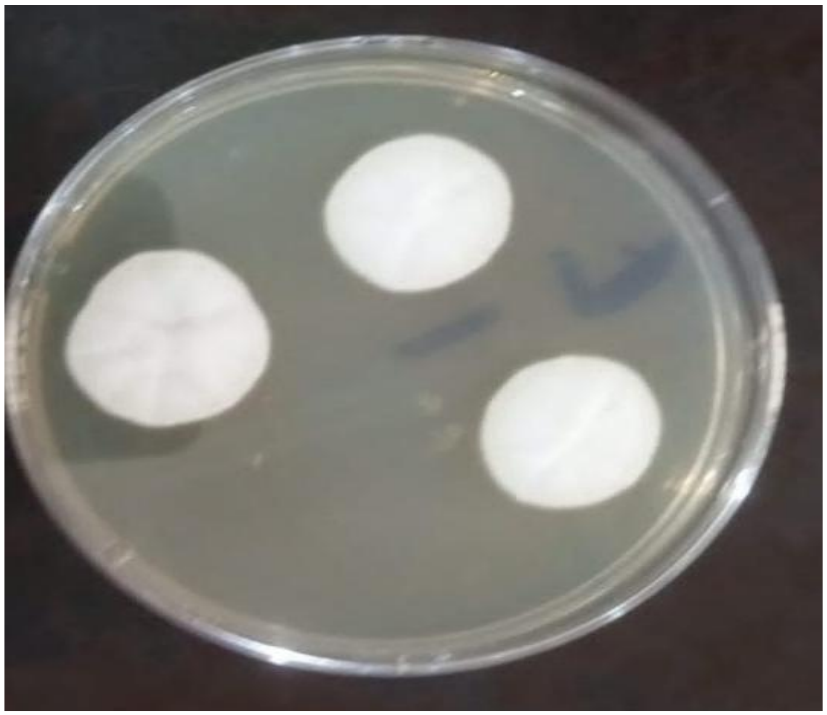

Plate 3. *Penicillium* sp. SGE33 on SDA.

Plate 4. *Aspergillius tritili* DTO438-H6 on SDA.

Table 2. Molecular identification and DNA sequencing result of isolates.

S/N	Description	Accession No.	% Relatedness
1	*Aspergillus flavus*	MH345909.1	99.82
2	*Aspergillus flavus* Strain ND63	MG659657.1	99.46
3	*Penicillium* sp. SGE33	JQ776535.1	99.64
4	*Aspergillus tritili* DTO438-H6	MZ014552.1	99.46

Table 3. Aflatoxin result of uncooked ofada rice using LC-MS/MS.

Analyte	Result	Units	Analysis date	Reporting limit (ppb)	Method	References
Aflatoxin B1	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168
Aflatoxin B2	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168
Aflatoxin G1	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168
Aflatoxin G2	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168

<RL = Less than reporting limit.

Table 4. Aflatoxin result of cooked ofada rice using LC-MS/MS.

Analyte	Result	Units	Analysis date	Reporting limit (ppb)	Method	References
Aflatoxin B1	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168
Aflatoxin B2	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168
Aflatoxin G1	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168
Aflatoxin G2	<RL	ppb	04/08/2024	1	LC-MS/MS	Internal SOP 14-168

<RL = Less than reporting limit.

where dust and fungal spores are prevalent, may also be a contributing factor, as is common in overcrowded areas. In addition, the common practice of storing rice in bags or open containers under humid conditions facilitates fungal invasion and colonization (Hell et al., 2000). Market practices, such as exposing grains to open air, stacking bags directly on damp floors, and lack of regular inspection further increase the risk of contamination. The total mold counts for the cooked rice ranged from $4.0x10^1$ in Victoria Island to $2.9x10^3$ in Mushin market. This reduction in total counts after cooking was also observed in other market locations (Table 1), However, statistical analysis by ANOVA revealed no significant difference ($p<0.05$) in mold counts between cooked and uncooked ofada rice. Breidt and Costilow (2004) stated that molds including spores are easily destroyed by heat treatment at 60 -71^{o}C. In this study, this assertion appeared to be correct but with no significant difference statistically. Molecular characterization identified the molds present in ofada rice as *Aspergillus flavus* (two different strains), *Aspergillus tritili* and *Penicillium* sp SGE33 (Table 2). *A. flavus* is a known producer of Aflatoxin. However, the aflatoxins analysed by LC-MS/MS for both the cooked and uncooked samples showed they were both less than the reporting limit of (<RL) of 1ppb. This is below the EU regulatory limits of 2ppb for Aflatoxin B1, indicating the probability of the presence of aflatoxins but in lower concentrations which could be due to the fermentation step involved in ofada rice production.

Role of fermentation in mitigating aflatoxin production

Fermentation process has been found to have a positive effect on fungal growth and mycotoxin production in foods. A study by Adewunmi and Chingoma (2023) showed that fermentation process is capable of reducing the mycotoxin content in fermented foods. They reported an 86% reduction in aflatoxin B1 and 66% reduction in Aflatoxin B2 levels in fermented grains after 96 h of fermentation compared to non-fermented grains. Another study by Oluwafemi and Ikeowa (2021) showed a 70% reduction in aflatoxin B1 in Ofada rice after 48 h of fermentation. A study by Adetunji et al. (2020) reported an 80% reduction in aflatoxin B1 and B2 using lactic acid bacteria and *Saccharomyces cerevisiae* as fermenting organisms over 72 h. This reduction in aflatoxin content

during fermentation could have been due to several reasons. Firstly, during fermentation, organic acids are produced which makes the environment very acidic for the growth of some toxigenic fungi, thus hindering their toxin production into the food. Daou et al. (2021) and Adewunmi and Chingoma (2023) posit that many mycotoxin producing fungi are sensitive to low pH conditions, and the acidification can inhibit their growth and mycotoxin production. Secondly, during fermentation, desirable microorganisms such as lactic acid bacteria, and yeasts can outcompete toxin-producing moulds, thereby reducing mycotoxin contamination in the fermented grains (Adebiyi et al., 2019). Thirdly, lactic acid bacteria which are usually present during fermentation can produce enzymes that can modify mycotoxin structures and reduce their toxicity (Adebiyi et al., 2019). In this study, two different species of *A. flavus* were isolated from Ofada rice, but with low concentrations of aflatoxins. This shows that aflatoxin production from this fungus may have been mitigated during the fermentation step in ofada rice production. This low concentration of aflatoxin could not be detected within the reporting limit of <1ppb.

Cooking as a secondary risk -reduction strategy

Although mold counts decreased after cooking (but not statistically significant), aflatoxin levels were not shown to change. While molds are easily destroyed by heat, mycotoxins are generally believed to be heat-resistant. However, a study by Karlovsky et al. (2016) posits that thermal processing has been used as mitigation strategy to reduce mycotoxins content in cereals. However, there is lack of information on the impact of domestic cooking on mycotoxins stability in foods. On the contrary, Sobral et al. (2019) stated that cooking can reduce mycotoxins content, although the impact differed among mycotoxins. Therefore, cooking can be a secondary risk-reduction strategy.

Conclusion

In this study, aflatoxins were not detected in both cooked and uncooked ofada rice. All samples were less than the reporting limit of 1ppb and the EU regulatory limit of 2ppb for AFB1. The rationale behind setting regulatory limits for aflatoxins and other mycotoxins in food products, including cereals, is to protect consumers. Low levels of aflatoxins in ofada rice in this study, irrespective of the presence of toxigenic *Aspergillus* species, indicate that this cereal is generally safe for consumption, as it poses no food safety or public health risks.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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