

EFFECT OF DIFFERENT STORAGE CONDITIONS ON AFLATOXIN PRODUCTION IN OVEN-DRIED TILAPIA FISH (*Oreochromis niloticus*)

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ABSTRACT

Aflatoxin produced by *Aspergillus* species are carcinogenic and pose significant health risks to consumers of contaminated food products. The storage conditions of oven-dried fish play a crucial role in controlling aflatoxin production. This study examined aflatoxin production in oven-dried tilapia fish under different storage conditions. A total of 36 fishes were bought fresh, oven-dried and stored in bamboo baskets and plastic containers. The isolation and Identification of fungi were carried out weekly for 4 weeks and aflatoxin was detected by high-performance liquid chromatography (HPLC) method. The highest moisture content (%MC) of the oven-dried Tilapia fish were subjected to temperature ranges 80 °C - 130 °C was 76.7% at 130 °C. The total fungal count ranges from 2.65±0.01 to 3.31±0.02 log cfu/g with the highest count recorded in covered bamboo basket while the lowest was from tightly covered storage plastic. Six fungal isolates namely *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *mucor*, yeast, and *Penicillium chrysogenum* were associated with the stored oven-dried tilapia fish samples. The oven-dried tilapia fish stored in bamboo baskets (opened and closed); and opened plastic container were contaminated with three (3) *Aspergillus sp.* and a *Penicillium sp.* with aflatoxin B1 levels ranging from 0.62 to 0.90 ppb. Remarkably, no fungi or aflatoxin were detected in the tightly covered storage plastics (TCSPs) containing fish that were oven-dried at 130 °C (designated as A) in sharp contrast to bamboo basket-stored fish which exhibited elevated fungal counts. The results obtained from this study highlights the critical function of storage conditions in controlling aflatoxin production in oven-dried tilapia fish. The application of best storage practices can decrease contamination, ensuring safer consumption and reduced health risks.

Key words: aflatoxin, oven-dried tilapia fish, storage conditions, food safety.

INTRODUCTION

Various fungal species, including *Aspergillus*, *Fusarium*, and *Penicillium*, are responsible for synthesizing mycotoxins of substantial importance, as elucidated by Osibona *et al.* (2018), Akwuobu *et al.* (2019), and Kusmarwati *et al.* (2021). These mycotoxigenic fungi pose a significant threat to food products across diverse stages of production and processing, especially under conditions characterized by elevated heat and moisture. The inherent stability of mycotoxins presents a formidable challenge for their removal from food items once they have permeated the product. The susceptibility of food

to mycotoxins is contingent on various influencing factors, including climatic conditions, storage standards, and harvesting methods. Research findings from Ferreira *et al.* (2019) and Dai *et al.* (2022) have identified independent physical, chemical, and biological factors that impact mycotoxin production.

Aflatoxins, acknowledged as a family of highly toxic and biologically active cancerous secondary metabolites, were first identified 43 years ago during an outbreak in England that resulted in the death of 100,000 turkeys, as chronicled by Lalah *et al.* (2020). These toxins, primarily originating from

Aspergillus flavus and *Aspergillus parasiticus*, contaminate a wide array of agricultural products, including peanuts, maize grains, cereals, spices, oils, fruits, milk, meat, vegetables, and animal feeds. Aflatoxins are also detected in animal products such as fish and insects, as reported by Oku and Amakoromo (2013).

The most pivotal mycotoxins encompass Aflatoxins (AF), Zearalenone (ZEA), Fumonisin (FB), Deoxynivalenol (DON), Patulin, Trichothecene, T2-toxin (T2), and Sterigmatocystin, as underscored by Awuchi *et al.* (2021). Within the category of aflatoxins, Aflatoxin B1 (AFB1) and Aflatoxin B2 (AFB2) are considered the most toxic and hazardous. Consumption or ingestion of mycotoxins can result in various health issues, including vomiting, abdominal pains, pulmonary edema, coma, convulsions, carcinogenic effects, loss of appetite, weight loss, faintness, depression, and even death. Aflatoxins, comprising over one hundred identified mycotoxins, are complex contaminants in food, with six significant ones designated as B1, B2, G1, G2, M1, and M2, as indicated by Fagbohun and Lawal (2015), Rafli *et al.* (2018), and Ousman *et al.* (2019). Molecular distinctions are observed between these aflatoxin groups; for instance, the B-group (B1 and B2) features a cyclopentane ring, while the G-group (G1 and G2) contains the lactone ring. The B-group aflatoxins exhibit blue fluorescence, whereas the G-group displays yellow-green fluorescence under ultraviolet (UV) light, underscoring the significance of fluorescence in distinguishing between the two groups. Aflatoxins M1 and M2, found in milk, are hydroxylated forms of AFB1 and AFB2.

According to the International Agency for Research on Cancer (IARC), aflatoxins B1 and B2 have been classified as Group 1 carcinogens, signifying their recognition as substances with established carcinogenicity (Marchese *et al.*, 2018). Exposure to significant concentrations of these aflatoxins poses a considerable risk of inducing carcinogenic effects and acute toxicity or fatality in both humans and livestock. Previous research has established a link between prolonged consumption of aflatoxin-contaminated food and the onset of liver cancer. Additionally, it has been

observed that feeds contaminated with aflatoxin contribute to the metabolic biotransformation of AFB1 into aflatoxin M1, a substance classified as a potential human carcinogen and included in the IARC Group of cancer-producing substances (Marchese *et al.*, 2018).

Various traditional methods have been employed in the processing and preservation of fish, including notable ones such as smoke-drying and fermentation. Among these techniques, smoke-drying is particularly prevalent, leading to a significant consumption of fish in this preserved state. This age-old processing method utilizes a combination of salt and smoke to effectively reduce moisture content, thereby preventing microbial and enzymatic spoilage (Adeyeye, 2016; Nwaigwe, 2017). In areas lacking refrigeration facilities, smoke-dried fish is preferred due to its cost-effectiveness and simplicity. This method plays a role in extending the shelf-life of fish and mitigating deterioration (Wogu and Iyayi, 2011). Microbial contaminants, such as bacteria and fungi, often arise from suboptimal handling practices, exposure to air, the source of the fish, or other degrading substances (Adeyeye, 2016; Nwaigwe, 2017). Some studies have examined aflatoxin contamination in dried fish and feed (Osibona *et al.*, 2018; Samuel and Odunigba, 2015); limited research focuses on the effect of storage conditions on aflatoxin production. Thus, this study aimed to investigate the effects of different storage conditions on aflatoxin production in oven-dried tilapia fish and identify optimal storage parameters to enhance the fish's shelf-life, ensuring its safety for consumption.

MATERIALS AND METHODS

Sample collection and preparation

A total of thirty-six (36) fresh Tilapia fish samples were purchased from the market in Ijebu-Ife, Ogun State, Nigeria. They were procured in three separate batches and subsequently transported to the laboratory under aseptic conditions. To determine the percentage moisture content (%MC) of the fish samples, a preliminary set of protocols such as washing, cleaning, and weighing were used for sample preparation. Subsequently, the samples were subjected to oven-drying at controlled temperatures of 130°C, 100°C, and 80°C. After the drying process, the fish samples

were allowed to cool, weighed and organized into three groups, each comprising 12 fishes, as outlined in Table 1 and Figure 1.

$$MC (\%) = \frac{(W1 - W2) \times 100}{W1}$$

Where W1 = weight (g) of sample before oven-drying

W 2 = weight (g) of sample after oven-drying

Table 1. Groups of stored oven-dried fish at different temperatures.

Sets	Group 1 (130 °C)				Group 2 (100 °C)				Group 3 (80 °C)			
3	A	B	C	D	E	F	G	H	I	J	K	L
Fish/container												
Storage	TCSP	USP	CBB	UBB	TCSP	USP	CBB	UBB	TCSP	USP	CBB	UBB

*TCSP, tightly covered storage plastic; USP, uncovered storage plastic; CBB, covered bamboo basket; UBB, uncovered bamboo basket.



Figure 1. Storage of oven-dried Tilapia fish in plastic container and bamboo basket.

Isolation and Identification of fungi strains

Pour-plate method was used to obtain fungal counts every 7 days. A weight of 1 g of sample was homogenized in 9 ml sterile distilled water to achieve ten-fold dilution and this was cultured on the Potato Dextrose Agar (PDA). To safeguard against bacterial contamination, the PDA was enriched with chloramphenicol, an antibiotic. The inoculated PDA plates were incubated at a temperature of 27 °C for 3 - 7 d, following incubation, fungal colonies were isolated and subsequently sub-cultured to achieve a purified culture. The macroscopic and microscopic analyses of the fungal cultures such as colony morphology, mycelia, and spore characteristics were achieved according to Samuel and Odunigba (2015).

Extraction of aflatoxin

Aflatoxin extraction from fish muscle was conducted following the procedures outlined by Hassan *et al.* (2011) and Mohamed *et al.* (2017), with some modifications. 10g of each oven-dried

fish sample, was subjected to a 50:50 v/v acetone:water solution (50 mL). These preparations were homogenized using high-speed blending for 3 min. followed by 30 min of shaking and subsequent filtration through Whatman No. 1 filter paper. Purification of the mixtures included the addition of 40 mL of 10% NaCl, accompanied by 25 mL each of n-hexane and dichloromethane. The resultant extracts were collected and evaporated to dryness in opaque bottles. Standard concentrations of aflatoxins B1, B2, and G2 were prepared. To create working solutions, 1 mL of acetonitrile was injected into each vial to dissolve the aflatoxins, and these solutions were stored at 8 °C.

High-performance liquid chromatography (HPLC) analysis

The assessment of aflatoxin presence and levels in the samples were carried out according to methods described by Samuel and Odunigba (2015), with minor adjustments. About, 100 µL of chloroform was added to both the sample extract and the aflatoxin working standards, thoroughly mixed for 30 s and then filtered using a No. 4 Whatman filter paper. Then, approximately 900

μL of water: acetonitrile (9:1 v/v) was added and further mixed for another 30 s. The resulting mixture was filtered using a membrane filter and was degassed in an ultrasonic bath for 25 min prior to utilization. For aflatoxin analysis, 30 μL of the mixture was injected into an Agilent high-performance liquid chromatography (HPLC) column, which had been pre-conditioned with 5 mL methanol and 5 mL acetonitrile:water (9:1 v/v) for 15 min. Quantification was performed using a fluorescence detector.

RESULTS

Moisture content of the tilapia fish

The percentage moisture content (%MC) of Fresh Tilapia fish are shown in Table 2. The fish subjected to oven-drying at 130 °C displayed the highest moisture content, ranging from 72.8 % to 81.4% (mean = 76.7 %). In a decreasing order, oven-dried fish at 100°C exhibited a moisture loss of 67.6 % to 76.7% (mean = 72.6 %). Conversely, the oven-dried fish at 80°C demonstrated the lowest moisture loss at values ranging from 52.0 % to 81.7 % (mean = 65.6 %).

Table 2. Percentage moisture content (%MC) of fresh Tilapia fish at varying temperature ranges (°C).

	130 °C			100 °C			80 °C		
	Initial weight (g)	Final weight (g)	% MC*	Initial weight (g)	Final weight (g)	% MC	Initial weight (g)	Final weight (g)	% MC
1	72.0	18.1	74.9	72.5	18.3	74.8	86.8	35.6	59.0
2	64.4	16.3	74.7	81.4	21.9	73.1	80.9	27.9	65.5
3	73.2	17.5	76.1	65.2	19.7	69.8	69.0	23.4	66.1
4	73.3	14.7	80.0	72.3	22.2	69.3	71.0	21.9	69.1
5	70.1	14.4	79.5	73.5	21.6	70.6	81.3	31.5	62.3
6	72.8	17.8	75.5	93.5	30.3	67.6	80.8	37.3	53.8
7	76.7	20.3	73.5	83.5	25.1	69.9	59.3	21.6	63.6
8	77.6	21.1	72.8	73.6	18.6	74.3	73.0	26.0	64.4
9	75.0	16.8	77.6	63.2	14.7	76.7	84.6	40.6	52.0
10	73.1	13.6	81.4	76.8	19.2	75.0	59.5	22.7	71.5
11	84.7	21.4	74.7	68.5	16.4	76.1	56.2	12.5	77.8
12	70.5	14.0	80.1	73.0	18.8	74.3	54.5	10.0	81.7
Mean %			76.7			72.6			65.6

*% MC - percentage moisture content

Fungal growth progression on stored oven-dried Tilapia fish

In the first week of storage, there was no indications of fungal growth in the four containers of Group 1, containing dried fish processed at 130 °C. However, obvious fungal growth was detected by the second week (11th day) in container C (covered bamboo basket - CBB), some oily deposits and reduction in fish sizes were observed in containers A (tightly covered storage plastic - TCSP) and B (uncovered storage plastic -

USP) (Figure 1 and Table 1). At the third week, container B displayed fungal growth akin to that observed in container C, and container D (uncovered bamboo basket - UBB) showed discoloration in the fish samples.

In Group 2 (oven-dried fish at 100 °C) remarkable fungal growth was evident in containers F (USP) and H (UBB) by the third day of storage; accompanied by the production of oily substances and a reduction in size in container E (TCSP)

(Figure 1 and Table 1). At week 2, consistent fungal growth patterns were observed in all the containers. For fish samples in Group 3, consisting of dried fish at 80 °C, the results differed from those in Group 1 and 2. Bacterial growth became apparent in fish samples by the second day, and maggot development occurred by the third day, impacting fungal growth. Consequently, samples from Group 3 were excluded from the aflatoxins production assay.

The total fungal count (log cfu/g) of the stored oven-dried Tilapia fish

The total fungal count of tilapia fish subjected to oven drying at 130 °C revealed counts of 3.04 ± 0.02 log cfu/g in the USP (labeled B) and 3.30 ± 0.01 log cfu/g in the CBB (labeled C). For oven-dried tilapia fish at 100 °C, the recorded counts varied: 2.65 ± 0.01 log cfu/g in the TCSP, 3.19 ± 0.01 log cfu/g in the USP, 3.30 ± 0.03 log cfu/g in the CBB, and 3.31 ± 0.02 log cfu/g in the UBB (Table 2 and Table 3). It is noteworthy that the bamboo basket exhibited the highest fungal counts among the fish samples.

Table 3. The Total Fungal Count (log cfu/g) of the oven-dried Tilapia fish.

Samples (Group 1 and 2)	Fungal Count (log cfu/g)
A	NG*
B	3.04 ± 0.02
C	3.3 ± 0.01
D	NG
E	2.65 ± 0.01
F	3.19 ± 0.01
G	3.30 ± 0.03
H	3.31 ± 0.02

Legend: *NG - No Growth.

Fungal strains

A total of six (6) fungal isolates were obtained from the oven-dried tilapia fish stored under

different conditions. Based on the morphological features, these isolates belong to different species of fungi as shown in Table 4.

Table 4. Morphological characterization of the fungi from the oven-dried Tilapia fish.

Isolate code	Morphological features	Fungus Identified
OFR1	The growth observed manifested as small, creamy, or white colonies with a rhizoid and filamentous form. The surface exhibited a wrinkled texture, and the prevailing color was white, accompanied by filamentous mycelium. The growth adopted a round shape, characteristic of a yeast strain.	<i>Yeast</i>
OFR2	The characterized growth underwent swift development, initially appearing cottony to fluffy and white, transitioning to yellow, and ultimately acquiring a dark grey hue with sporangia formation. The coloration was predominantly white, featuring highly myceliated and branched hyphae. The surface presented a fluffy texture, and the growth displayed irregular non-septate hyphae, forming fine threads topped with ball-shaped structures—typical of <i>Mucor</i> .	<i>Mucor</i>
OFR3	The identified growth showcased a network of branching and fusing hyphae, forming a felt-like conidiophore structure. The color was black, with a white to yellow reverse. Smooth and colorless conidiophores and spores were present, and the surface exhibited a velvety, cottony, or smooth texture. The growth displayed a small, columnar globous shape, indicative of <i>Aspergillus niger</i> .	<i>Aspergillus niger</i>
OFR4	The discerned growth featured soft velvety colonies of yellowish-green color, characterized by visible molds and a thick mycelial/mat structure discernible to the naked eye. The shape was globose/sub-globose, consistent with <i>Aspergillus flavus</i> .	<i>Aspergillus flavus</i>
OFR5	The colonies initially appeared white and transformed into a blue to blue-green hue. The growth was rapid, flat, and exhibited a filamentous, velvety, woolly, or cottony texture. It displayed septate hyaline hyphae, along with simple or branched conidiophores, metulae, phialides, and conidia. The formation of brush-like clusters, referred to as "penicilli," identified this growth as <i>Penicillium sp.</i>	<i>Penicillium sp.</i>
OFR6	The observed colonies originated from conidiophores and displayed a grey-green color. The growth featured flask-shaped or cylindrical phialides, with deciduous and globose, oblong to elliptical conidia. The surface was smooth, and the growth exhibited filamentous conidia forming dense, intertwined mycelial structures—characteristic of <i>Aspergillus fumigatus</i> .	<i>Aspergillus fumigatus</i>

Aflatoxin production

A total of six samples, specifically B and C from Group 1, and E, F, G, and H from Group 2, were selected for aflatoxin screening. A single type of aflatoxin, identified as aflatoxin B1, was detected in the stored oven-dried fish samples. Aflatoxin

B1 was found in samples C, F, G, and H, while it was undetected in samples B and E (Table 5). The concentration of aflatoxin B1 varied among the samples, with the highest concentration observed in sample H and the lowest in sample C.

Table 5. The concentration of aflatoxin in the stored oven dried tilapia fish.

Samples (Group 1 and 2)	Aflatoxin B1 (ppb)
A	ND*
B	absent
C	0.62
D	absent
E	absent
F	0.83
G	0.75
H	0.90

*ND, Not Determined.

DISCUSSION

The findings from this study underscores the pivotal role of optimal storage conditions in mitigating the presence of storage fungi in dried fish products. Notably, fungal growth gradually progressed over time in oven-dried fish stored in TCSP containers labeled A and E. The efficacy of oxygen-deficient storage atmosphere, as fungi are primarily aerobic organisms, proved instrumental in reducing fungal invasion and aflatoxin (mycotoxin) production, aligning with earlier research by Samuel and Odunigba (2015). Furthermore, the study agrees with an earlier study which recommended criteria for selection of packaging materials with characteristics such as inertness, leak-proofing, impermeability to oxygen and moisture, low transparency, and resistance to abrasion and puncture (Osibona *et al.*, 2018).

The contamination of fish and related products by yeast and mold is contingent upon geographical location, moisture content, temperature, and hygienic conditions (Mohamed *et al.*, 2017). The application of the oven-drying methods, aimed at reducing moisture levels and extending storage duration, led to the observation of oily substances on the fish in container A due to elevated drying temperatures, effectively delaying the deterioration process and microbial growth. The initial microbial growth detected in the bamboo basket during the early weeks of storage reflect potential sub-optimal conditions, as air carrying spores of *Aspergillus flavus* from the environment through basket holes contributed to microbial growth. Importantly, the study clarified that microbial contamination on storage containers originated from the surrounding environment, as the fish itself did not exhibit fungal or microbial growth. Furthermore, the identified organisms on the fish were those capable of utilizing the fish and its substrates.

Total fungal counts within the range of 2.65 ± 0.01 – 3.31 ± 0.02 log cfu/g, as documented in this study, mirrored earlier reports by Job *et al.* (2016) regarding *A. flavus* contamination in smoked-dried fish and Demble *et al.* (2020) reporting analogous total fungal counts in smoked fish (*Clarias gariepinus*) in Mali. The six fungi species identified are *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus*

fumigatus, *Mucor*, yeast, and *Penicillium chrysogenum*. The prevalence of *Aspergillus* species, particularly *Aspergillus flavus*, aligned with findings from Osibona *et al.* (2018), Akwuobu *et al.* (2019), and Kusmarwati *et al.* (2021). Additionally, the predominance of filamentous fungi over yeast-like fungi in dried fish, attributed to their robust reproduction and diffusion capabilities, was consistent with observations in this study.

Aflatoxin B1 (AFB1) was detected in this study, with concentrations ranging from 0.62 to 0.90 ppb which was lower than the maximum amount recommended amount of 15 ppb by FAO. Despite this, AFB1 has been recognized as the most toxic among aflatoxins, poses potential health risks, including hepatocellular carcinoma upon chronic exposure. The study underscores the importance of enforcing for stringent regulations to ensure the quality and safety of dried fish products, given the widespread consumption of this popular food item, especially in coastal areas.

This study highlights the necessity of adhering to proper storage and packaging practices to prevent mycotoxin contamination in stored dried fish products. The findings have shown the importance of raising awareness about aflatoxins and associated health hazards linked to the consumption of mycotoxin-contaminated fish products. To safeguard public health, stringent regulations and the establishment of effective food safety management systems governing drying, storage, and sales environments in the dried fish industry are essential.

CONCLUSION

This study has established the effective methods that can prevent aflatoxin contamination of stored oven-dried fish products by using proper storage and packaging materials. This is necessary as the accumulation of aflatoxin can lead to serious health hazard to humans as the final consumer of the stored dried fish products. Therefore, there should be more awareness to the general public on aflatoxins and the dangers associated with the consumption contaminated fish products. It is also important that guidelines for safe storage and handling practices be developed by appropriate agency.

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CONFLICT OF INTEREST

The authors declare no conflict of interest affiliated to this article

AUTHORS CONTRIBUTION

Ogundipe O. R.: Investigation, Methodology, Data Analysis,
 Feruke-Bello, Y. M. and Okeleye, B. I.:
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