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FUNGAL CONTAMINATION RISKS IN TRADITIONAL AGIDI: A FOOD SAFETY CONCERN IN NIGERIA

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Abstract
Agidi, a popular maize-based delicacy in Nigeria, is highly prone to fungal contamination due to its high moisture content and neutral pH, posing serious health risks to consumers. Proper handling and preservation are vital, as contamination with toxic fungi like Aspergillus niger can lead to mycotoxin exposure, causing foodborne illnesses and long-term health concernsThis study aimed to isolate and identify fungal species associated with spoilt agidi and assess the biochemical changes resulting from fungal spoilage. Spoilt agidi samples were cultured on Potato Dextrose Agar and molecularly characterized using ITS primers, identifying *Trametes polyzona* and *Aspergillus niger* as major fungi implicated in spoilage. Analysis of fresh and spoilt samples revealed significant declines in nutritional parameters, including protein (0.50 ± 0.02% in fresh vs. reduced in spoilt), crude fibre (3.99 ± 0.02% in fresh), crude fat (0.61 ± 0.01% in fresh), vitamins A, B-complex, C, and E, essential amino acids (threonine and valine), and minerals (calcium, iron, and zinc) in spoilt samples ($p < 0.05$). The moisture content increased significantly in spoilt samples (20.75 ± 0.88%). These findings highlight the need for strict hygienic measures, improved storage conditions, and natural antifungal agents to prevent spoilage, extend shelf life, and ensure the safety of agidi.
Keywords: Agidi, *Aspergillus niger,* Fungal Contamination, Food Safety, Spoilage Prevention

INTRODUCTION

In Nigeria, a plate of warm agidi can be a comforting staple, but lurking beneath its soft texture and delicious taste is a hidden danger - fungal contamination. Agidi also known as maize pudding, a traditional maize-based delicacy, is highly susceptible to fungal growth due to its high moisture content and neutral pH, posing significant health risks to millions of consumers (Pitt & Hocking, 2019). According to Nwankwo and Okereke (2021), perishable food is susceptible to fungal contamination during fermentation and storage, jeopardising its safety and quality. With a lifespan of only 3-4 days, fungal development can lead to spoilage and mycotoxin generation, posing dangers to health (Adebayo & Aderiye, 2018).

Several fungal species have been identified as being responsible for maize pudding spoiling, including *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer, Paecilomyces variotii, Penicillium crustosum,* and *Trichoderma harzianum* (Adeogun *et al.,* 2018).

Despite its popularity, the fungal species driving this spoilage and the resulting biochemical changes remain poorly understood. Culture-based identification methods often lack specificity, emphasizing the need for molecular techniques like ITS sequencing (Schoch *et al.,* 2012). This study isolates and identifies fungal species in spoilt maize pudding using molecular characterization, assessing implications for food safety and informing control strategies to improve quality and safeguard consumer health. Effective control measures are crucial to mitigate fungal contamination and ensure the safety of this widely consumed traditional food in Nigeria.

MATERIAL AND METHODS

Study Area

This research was carried out at the Mycology/Pathology laboratory of Plant Science and Biotechnology and the Regional Centre for Biotechnology and Bio-fuel Research Laboratory, University of Port Harcourt, where DNA extraction took place and the biochemical analysis (IITA, Ibadan), between February 1st, 2025, and September 30th, 2025.

Materials

Source of plant materials

Fresh agidi samples (n=5, replicated in triplicate) were collected from a Port Harcourt vendor (Feb 2025).

Isolation of fungi from staled agidi using direct plating method

Fungal pathogens were isolated from spoilt agidi using a modified direct plating method (Ikechi-Nwogu & Amos, 2020). Agidi samples were placed on sterile PDA plates, incubated at 25±2°C for 5-7 days, and sub-cultured on PDA for identification.

Proximate Analysis: Proximate analysis (AOAC, 1990) determined the nutritional composition of agidi, including moisture, ash, protein, carbohydrates, and lipids. Crude fiber was calculated indirectly by subtraction.

Determination of Vitamins: Vitamins A, B1, B2, B3, B6, B9, B12, E, and K were extracted and determined using Okonwu *et al.*'s method (2018a, b). Vitamin C

was determined using titrimetric method (Okwu, 2004). In Vitamin A Extraction Method, 0.5g plant sample + 20ml 0.2N HCl (1.5 hours) → pH adjusted to 4.5 → centrifuged → supernatant mixed with acetic acid, H2O2, sodium hydrogen sulphate → HPLC analysis. And in Vitamin B Extraction Process, Sample Prep: 2.5g plant sample + extraction solution (water: HCl:0.1N H2SO4, 5:2:3) Extraction: Warmed (40°C, 2 hours) → Cooled → Centrifuged

Determination of Crude Protein (Kjeldahl method): Nitrogen content was determined using Kjeldahl method. Plant samples (0.1g) were digested with sulfuric acid and catalyst, then distilled and titrated with 0.1N HCl. %Nitrogen = (Titre value x 1.4 x 100) / (1000 x 20 x 0.1). Crude protein = %Nitrogen x 6.25.

Determination of Carbohydrate (Clegg Anthrone method): Carbohydrate (CHO) content was determined using Anthrone method. Plant samples (1g) were hydrolysed with perchloric acid, filtered, and reacted with Anthrone reagent. Absorbance was read at 630nm. %CHO = (25 x Absorbance of sample) / (Absorbance of standard glucose x 1g sample).

Determination of Moisture using the Air Oven Method: Moisture content was determined by oven drying (105°C, 6 hours). % Moisture = [(fresh weight - dry weight) / fresh weight] x 100.

Determination of Lipid by Soxhlet Extraction Method: Lipid content was determined using Soxhlet extraction with acetone. % Lipid = [(weight of flask + extract - weight of flask) / sample weight] x 100.

Determination of Ash by Furnace Method: Ash content was determined by incineration (630°C, 3 hours). % Ash = [(weight of crucible + ash - weight of crucible) / sample weight] x 100.

Determination of Fibre: For crude fiber, it typically involves defatting the sample, digestion with acid (e.g., H2SO4) and alkali (e.g., NaOH), filtering and weighing the residue, ashing to determine mineral content, and calculating fiber content by difference.

Vitamin Composition: Vitamins were determined using various methods:

- A, E, C, B12: AOAC (2010) and Achikanu *et al.* (2013)
- B1, B3: Okwu and Ndu (2006)
- B2, K, Folate: Okwu and Josiah (2006)
- β-carotene: Oulai *et al.* (2014)

Determination of Amino Acids Using Water 616/626 LC (HPLC) Instrument:

The sample preparation and determination were carried out in the following four stages:

***Hydrolysis*:** Sample preparation involved hydrolysis (0.5g, 20-23 hours, 108°C, 10.05N HCl) with Norleucine as internal standard. Hydrolysates were dissolved in EDTA-containing water and stored in HPLC bottles for analysis.

***Derivatisation*:** hydrolysed samples reacted with phenyl isothiocyanate (PITC) to form PTC-amino acid derivatives (45 minutes). Standards (0-2.0 µmol) were also derivatized. Separation was done on Waters 616/626 HPLC.

***HPLC separation and Quantization*:** PTC-amino acids were separated on C18 column (30 minutes, 254nm detection). Buffer system: A (140mM sodium acetate, pH 5.5) and B (80% acetonitrile), gradient elution (0-55% B).

***Data Processing / Interpretion and Calculation*:** Dionex Chromeleon software calculated amino acid amounts (pmol) using response factors from standards. Results were normalized to internal standard (Norleucine) and expressed in mg/ml.

Mineral Analyses

- AAS (Mg, Zn, Fe, Cd, Cu, Pb, Ca, Ni, P): ash solution (5g, 550°C, 5hrs, 100ml deionized water)
- Flame photometer (K, Na): Jenway Digital with element-specific filters
- Calibration: standards from metal salts, acetylene gas for AAS

RESULTS

Isolation, morphological and microscopic identification of fungi associated with maize pudding

The result of the fungal isolation is presented in plate 1a and b Two unidentified fungal organisms AB5 and AB8 were isolated and found to be associated with maize pudding (Plate 1a and b). This study investigated the molecular characterization of the fungus associated with maize pudding. Macroscopically, the growth of the isolate AB 8 initially appeared white but transformed into dark black with visible spores after a few days. Based on the photomicrograph, the isolate was identified as a species of *Aspergillus*. AB 5 typically exhibited characteristics like fluffy or cottony texture that appeared white to cream-colored initially.

Plate 1a: Pure Cultures of Fungal Sample 2B Isolated from Spoilt Maize pudding on Potatoes Dextrose Agar 1b: micro-morphological presentation of isolate.

DNA Successfully Isolated from Fungal Cultures: The DNA isolation process produced high-quality genomic DNA from all fungal samples. Spectrophotometric analysis revealed concentrations of 65.3 ng/µL and 45.3 ng/µL for isolates AB5 and AB8, respectively. Agarose gel electrophoresis showed distinct, sharp bands, confirming the integrity and purity of the extracted DNA.

Fungal DNA Successfully Detected via PCR: The ITS PCR reactions were successful in amplifying the targeted fungi ITS region across all samples. Agarose gel electrophoresis of the PCR products revealed distinct bands at the expected sizes of ~200-420bp (Fig.1) for ITS genes. The uniformity and intensity of the bands indicated robust and consistent amplification using FirePol Master Mix. Gel electrophoresis results confirmed the quality and size of the PCR products. Bands corresponding to the expected sizes were observed, validating the specificity of the amplification.

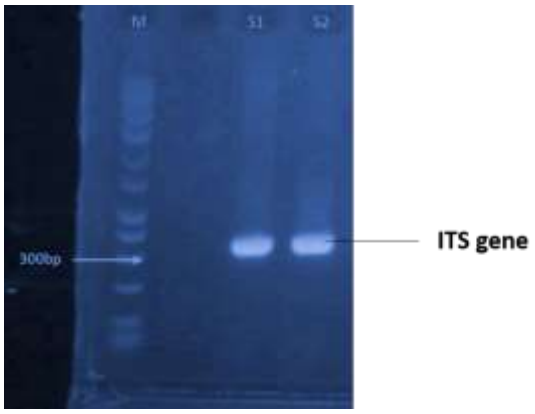

Figure 1: Amplified PCR Product Generated from Isolates Lane M: 100 bp ladder, S1 (AB5): ~300 bp, *Trametes polyzona,* S2 (AB8): ~300 bp, *Aspergillus niger* complex

Fungal Species Identified via Sanger Sequencing

Sanger sequencing of the PCR amplicons produced high-quality chromatograms with well-defined peaks. The sequencing outputs were processed and analysed using bioinformatics tools, and subsequent BLAST searches against reference databases enabled the identification of the fungal species present in each isolate.

BLAST result and phylogenetic tree construction

The BLAST analysis of the two query sequences revealed distinct fungal origins from separate lineages.

- **AB5:** *Trametes polyzona* (Basidiomycota), showing 98% identity, E-value: 9.0 × 10^-105
- **AB8:** *Aspergillus niger* (Ascomycota), showing 91-92% identity, E-value: 1.0 × 10^-83 to 5.0 × 10^-82.

Collectively, these findings provide strong evidence for the accurate identification of the fungal isolates and highlight their potential ecological and biotechnological relevance.

Figure 2: Phylogenetic Placement of Isolate AB5

Figure 3: Phylogenetic Placement of Isolate AB8

Biochemical changes in fresh and spoilt agidi were also observed and the results are shown below

Proximate Composition

The proximate composition of fresh and spoilt agidi shows significant differences (Fig 4). Fresh agidi has lower moisture content (17.25%) compared to spoilt agidi (20.75%). However, protein, ash, crude fibre, crude fat, and nitrogen are almost twice as high in fresh samples. This indicates fungal enzymatic activity breaks down nutrients, increasing moisture and depleting others. Statistical findings support this ($F = 1682.45$, $p < 0.001$).

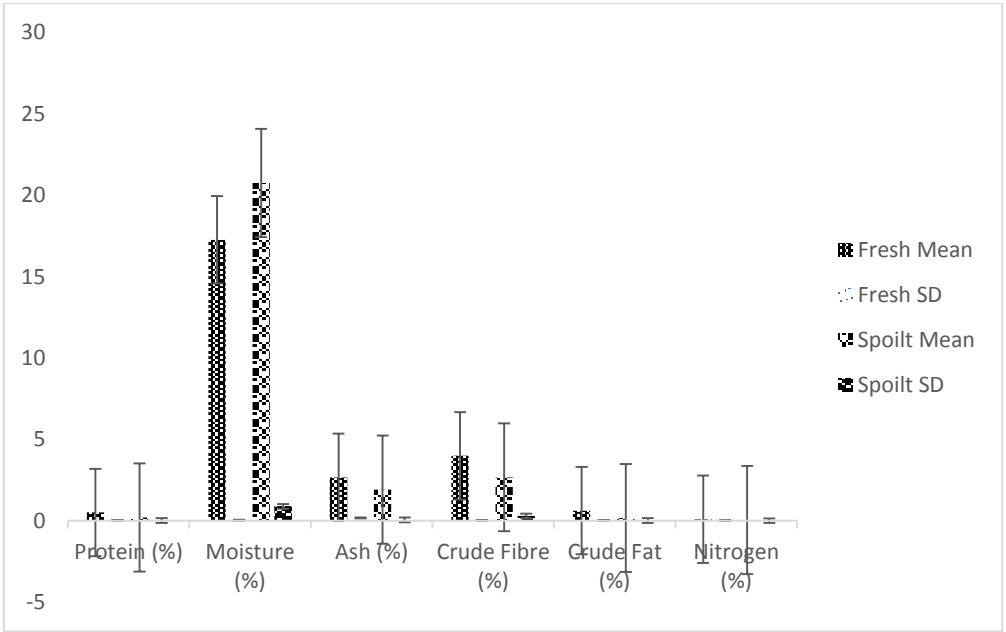

Figure 4: Proximate composition of fresh and Spoilt Agidi

Detectable Vitamins

The vitamin content (Fig 5) in fresh agidi is 2.84%, way higher than the 1.10% in spoilt agidi, showing a reduction of over 60%. This suggests fungal contamination leads to significant vitamin degradation. Among the vitamins analysed, Vitamin A (1.79 ± 0.07%) was the most abundant, followed by Vitamin B_3 (0.50 ± 0.02%), while Vitamin E (0.021 ± 0.001%) was the least abundant in the fresh sample. In the spoilt agidi, Vitamin A (0.69 ± 0.02%) and Vitamin B_3 (0.19 ± 0.01%) remained the highest, though markedly reduced in concentration. Statistical analysis revealed highly significant differences ($p < 0.001$) across all vitamins, with Vitamin E ($F = 1803.47$) and Vitamin B_2 ($F = 1861.84$) showing the largest variations.

The marked decline in both fat- and water-soluble vitamins reflects oxidative degradation and microbial utilization during fungal metabolism. Fat-soluble vitamins (A and E) are prone to oxidation, while water-soluble vitamins (B complex and C) are depleted rapidly as cofactors in fungal enzyme systems.

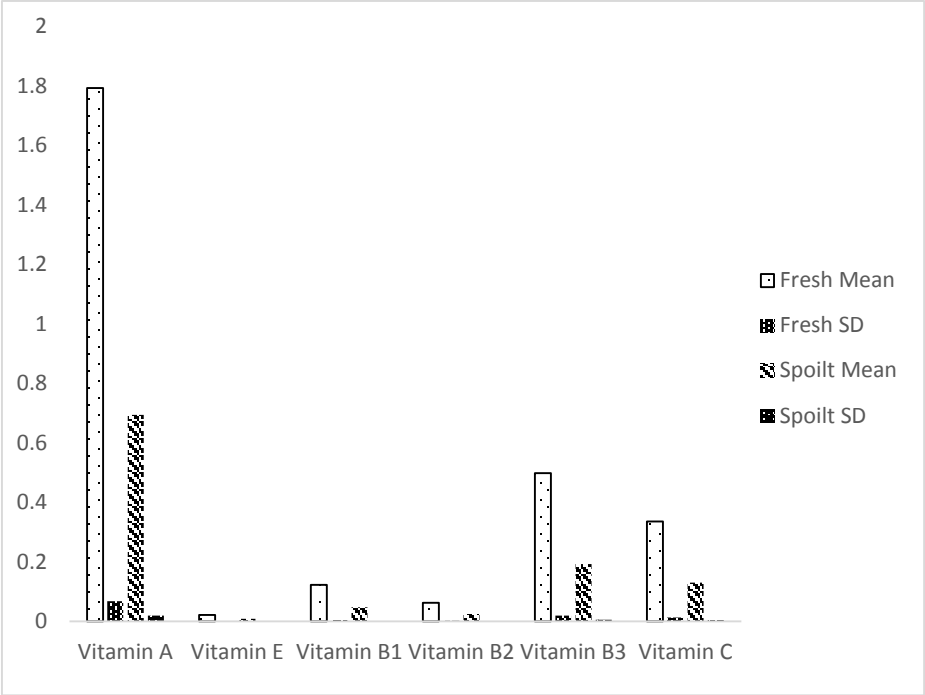

Figure 5: Vitamin content of fresh and spoilt agidi

Amino Acids

Twenty amino acids were assessed and quantified in Fresh and spoilt agidi. The bar chart (Figure 6) below shows the decline in most essential amino acids in contaminated samples compared to fresh agidi. Fungal spoilage reduced agidi's amino acids: EAA from 12.50% to 5.60%, NEAA from 3.60% to 2.20%. Threonine and valine were most abundant. Significant reductions ($p < 0.001$) due to fungal enzymes breaking down proteins

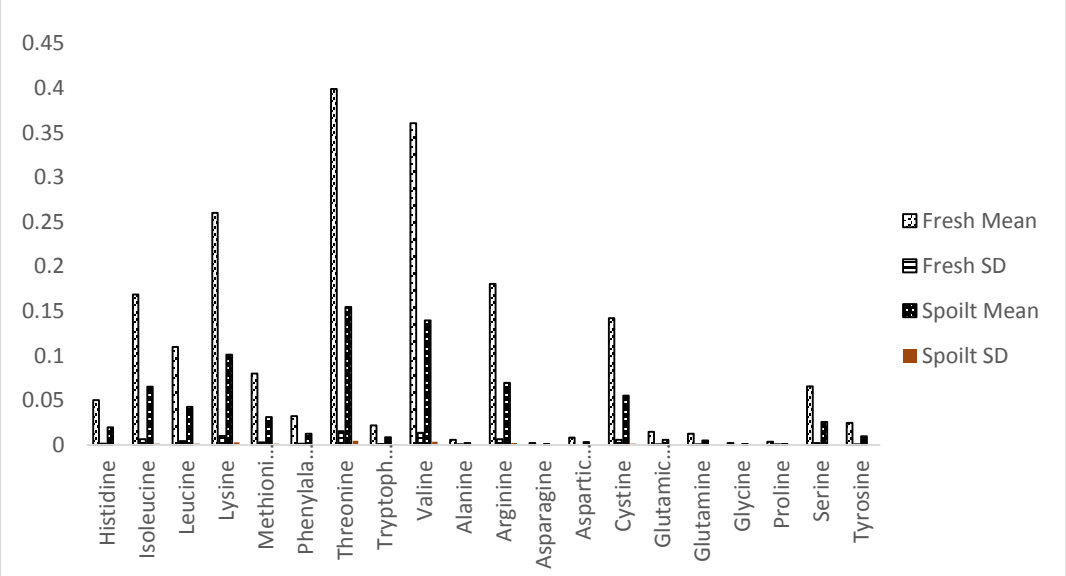

Figure 6: Amino acids and concentrations in fresh and spoilt Agidi

Mineral Composition

The minerals assessed in fresh and spoilt agidi and its concentrations are presented in (Figure 7). The mineral content of fresh agidi is higher than spoilt ones, except for sodium. Fresh agidi has more calcium, magnesium, potassium, manganese, iron, zinc, and copper. Statistical analysis shows significant differences ($F = 1152.78$, $p < 0.001$), indicating fungal contamination affects mineral composition.

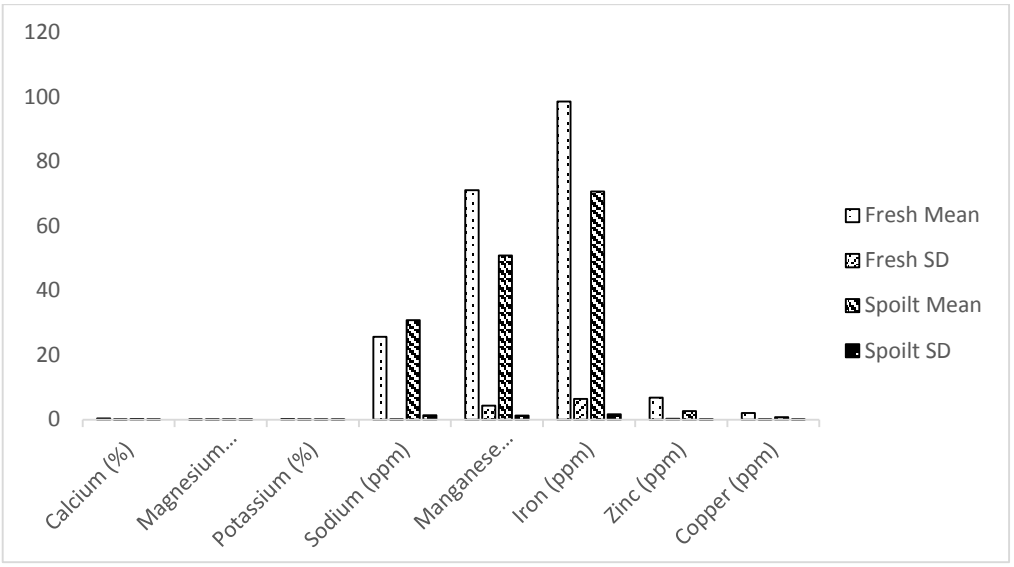

Figure 7: Mineral composition of fresh and spoilt agidi

DISCUSSION

This study identified *Aspergillus niger* and *Trametes polyzona* as fungi causing agidi spoilage using morphological and molecular techniques (ITS sequencing). Traditional methods were less reliable due to environmental influences. Fungal contamination significantly degrades maize pudding (agidi)'s nutritional value: protein, fat, ash, fibre, and nitrogen decrease, while moisture increases from 17.25% to 20.75%. Vitamins A, E, B-complex, and C drop by approximately 60-65%. Essential and non-essential amino acids also decrease. Key minerals like calcium, magnesium, and iron decline, except sodium which slightly increases. Fungal enzymes break down nutrients for growth, reducing agidi's nutritional quality. Various fungus species, such as *Aspergillus, Penicillium, Fusarium, Alternaria,* and *Mucor*, are known to cause food spoilage, thriving in specific substrates and climates (Dijksterhuis, 2019). Interestingly, our findings reveal that agidi is contaminated with *Aspergillus niger* and *Trametes polyzona,* echoing the results of Agwu (2021), who detected similar fungal contaminants in ready-to-eat agidi. Furthermore, our results align with Ajayi and Asunbo (2019), who identified *Aspergillus* spp. as the predominant fungus in agidi, alongside other species like *Saccharomyces cerevisiae* and *Fusarium* spp. These findings are also consistent with Adebayo *et al.* (2019), who isolated *Aspergillus niger* and *Penicillium chrysogenum* in maize pudding, highlighting the prevalence of these fungi in similar food products.

Aspergillus niger, a member of the phylum Ascomycota and family Trichocomaceae, is a ubiquitous saprophytic fungus thriving in various environments, including soil, air, and decaying vegetation (Kothari & Goyal, 2013). Its presence in maize pudding suggests post-processing contamination, likely due to inadequate hygiene, airborne spores, or contaminated equipment and packaging. This finding aligns with Nwankwo *et al.* (2021), who identified *A. niger* as a prevalent contaminant in spoiled maize pudding, and Okafor *et al.* (2018), who found *Aspergillus* species dominant in fermented maize-based foods in Nigeria, facilitated by high humidity and warm temperatures

Aspergillus niger's adaptability and ability to utilize various nutrients make it well-suited to thrive on maize pudding, producing enzymes like amylases, lipases, and proteases that break down starch, lipids, and proteins, leading to spoilage symptoms like softening, discoloration, and off-odors. This is consistent with Adeogun *et al.* (2018), who reported *Aspergillus* species degrade maize starch and protein in fermented foods. *A. niger's* thermotolerance and xerophilic nature allow it to grow in low-moisture environments and survive mild heating, making it resilient in ready-to-eat products stored at ambient temperatures (Bennett & Klich, 2003). Once established, it proliferates rapidly, forming dense mycelia and producing abundant spores, facilitating further contamination. This explains why *Aspergillus* species are often predominant in studies on fungal spoilage of traditional foods (Olowe *et al.,* 2023) Beyond its role in spoilage, *Aspergillus niger* poses significant public health concerns due to its production of mycotoxins like ochratoxin A and fumonisins, which are linked to nephrotoxicity, immunosuppression, and carcinogenesis (Bennett & Klich, 2003). Chronic exposure to these toxins can lead to serious health issues, including liver cancer and kidney dysfunction. The presence of *A. niger* in maize pudding is particularly alarming since it's consumed without further heating, allowing toxins to remain intact. Afolabi *et al.* (2020) highlighted the risks of toxigenic *Aspergillus* species in ready-to-eat foods in Nigeria, emphasizing the need for improved hygiene. Additionally, the identification of *Trametes polyzona*, a white-rot fungus typically associated with decaying wood, suggests environmental contamination, possibly from contaminated surfaces or air during processing (Oghenetega *et al.,* 2020).

The detection of *Trametes polyzona*, although not typically implicated in food spoilage, highlights poor environmental hygiene during production. This white-rot fungus secretes lignin-degrading enzymes, making it more suited to decomposing complex plant polymers than starch-based substrates (Baldrian, 2006). In contrast, *Aspergillus niger* is the primary spoilage organism driving biochemical and physical deterioration of maize pudding. The co-isolation of *A. niger* and *T. polyzona* illustrates the diversity of fungal flora introduced through unsanitary handling and open-air vending. This mixed fungal population mirrors Magan and Evans' (2000) observation that one species often dominates as a primary colonizer, while others coexist as secondary or environmental contaminants. Here, *A. niger* takes centre stage, with *T. polyzona* playing a passive role, showcasing the complex ecological interactions in food deterioration.

The presence of these fungi highlights the need for strict hygienic control during and after maize pudding preparation. *Aspergillus niger's* resilience and mycotoxin production mean poor handling, high humidity, and lack of refrigeration amplify fungal proliferation risks. These findings align with Nwankwo *et al.* (2021) and Okafor *et al.* (2018), who linked inadequate sanitation and open-air exposure to fungal contamination. Implementing good manufacturing practices, clean utensils, proper packaging, and controlled storage can reduce contamination and extend shelf life.

The use of molecular techniques, specifically ITS sequencing, provided accurate species-level identification, overcoming morphological method limitations (Glass & Donaldson, 1995). This approach enhanced finding reliability and sets a baseline for future Nigerian food studies.

In summary, *Aspergillus niger* drives maize pudding spoilage, while *Trametes polyzona* is an environmental contaminant.

Proximate Composition

The study revealed a notable increase in moisture content - spoilt agidi had 20.75% moisture compared to 17.25% in fresh agidi. This rise is attributed to fungal growth, which boosts moisture levels through; Respiration (producing metabolic water, increasing humidity) and enzyme breakdown (releasing bound water from complex molecules (Dijksterhuis and Houbraken, 2025). Together, these processes increase the overall moisture content.

The reverse was the case for protein, ash content, crude fibre, crude fat, and nitrogen. This result is in conformity with that of Hocking (2016), who reported that fungal development reduces the amount of protein, crude fibre, crude fat, and nitrogen by converting these components into nutrients and energy for their own metabolic activities and growth

The total vitamin content of agidi took a drastic hit, plummeting by over 60% from 2.84% in fresh samples to just 1.10% in spoilt ones. This significant decline is largely due to fungal activity, as fungi utilize vitamins as crucial elements for their growth and metabolic processes (Al-Shihkly *et al.,* 2009). Similar tendencies have been observed in other spoilt foods, where fungus enzymes destroy vitamins, making them inaccessible (Gharibzahedi *et al.,* 2014).

Fungal decay decreased agidi's amino acids by approximately 55.2% for EAA and 38.9% for NEAA. The lines of evidence show that amino acids play a critical role in fungal growth and reproduction, which is supported by Zain *et al.* (2024).

Fungal contamination significantly impacted mineral composition ($F = 1152.78$, $p < 0.001$), reducing essential minerals like calcium, iron, zinc, and manganese. Omemu *et al.* (2017), reported similar findings.

The decline in nutritional quality and increased fungal contamination in agidi pose health risks, especially for vulnerable groups like children, pregnant women, and the elderly. Deficiencies in amino acids, vitamins, and minerals may lead to health issues, while fungal growth can produce mycotoxins associated with allergic reactions and cancer. To mitigate these risks, it is essential to implement rigorous hygiene measures during processing and storage, including proper packaging and using natural antifungal agents, along with raising awareness about safe handling practices among stakeholders.

CONCLUSION

This study reveals that fungal contamination, particularly by *Aspergillus niger* and *Trametes polyzona,* significantly compromises the nutritional quality of agidi. The observed decline in protein, vitamins, amino acids, and minerals poses health risks to consumers, especially vulnerable populations. Implementing strict hygiene measures, proper storage, and using natural antifungal agents can mitigate these risks. Raising awareness among stakeholders about safe handling practices is crucial for ensuring the nutritional safety of agidi.

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