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Effects of steeping on fungal contamination and diversity in achi (*Barchystegia eurycoma*) seeds sold in some selected markets in Lagos state, Nigeria

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Abstract
Achi (*Brachystegia eurycoma*) seeds are widely used as a thickening agent in traditional Nigerian cuisine. However, the processing methods used for achi seeds can lead to fungal contamination, posing considerable health risks to consumers. This study aims to investigate the effect of water steeping on fungal species contaminating achi seeds sold in some selected markets in Lagos, Nigeria. The achi seeds were steeped by soaking them in water at 37 °C for two days, and the moisture content was subsequently determined. The isolated fungal species were identified using both morphological and molecular techniques. Our findings show that the moisture content (37.82—51.64%) of the steeped achi seeds was higher than that of the unsteeped achi seeds (8.32—15.73%). Seven fungal species were recovered from the achi seeds, including six *Aspergillus* spp. (*A. aculeatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. japonicus*, and *A. oryzae*), along with *Rhizopus microsporus*. Notably, the steeped samples were found to contain all the seven fungal species whereas the unsteeped achi were contaminated with just four fungal species, namely *A. aculeatus*, *A. flavus*, *A. fumigatus*, and *A. niger*. Additionally, the steeped achi seeds showed a higher fungal load (4.92×10^3—5.50×10^3 CFU/g) compared to the unsteeped samples (3.39×10^3—5.33×10^3 CFU/g). This current study indicates that steeping increases fungal contamination and diversity in achi seeds, raising concerns about food safety.

Keywords Achi seeds · Moisture content · Molecular identification · Fungal load · Fungal diversity · Food safety

1 Introduction

Achi (*Brachystegia eurycoma*) seeds, also known as African salad seeds, are small, flat, and disc-shaped seeds obtained from the African tree *Barchystegia eurycoma*. These seeds are commonly used in Nigerian cuisine, particularly in the preparation of soups and sauces, serving as a crucial ingredient in various local dishes, particularly as thickening agent [1, 2]. This seed offers a range of nutritional benefits, contributing to the overall health and well-being of individuals. Achi seed is a good source of plant-based proteins, carbohydrate, dietary fiber, and healthy fats, including omega-3 and omega-6 fatty acids [3, 4]. Furthermore, achi seeds are a good source of various vitamins and minerals, including vitamin

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A, vitamin C, calcium, phosphorus, and iron [3, 4]. These nutrients play vital roles in maintaining bone health, supporting the immune system, and preventing nutritional deficiencies. Achi seeds also contain phytochemical and antioxidants, which help protect the body's cells from damage caused by free radicals and lowering the risk of chronic diseases [5]. In addition to the plant nutritional properties, some traditional medicine systems attribute medicinal properties to achi seeds. They are believed to have anti-inflammatory, anti-cancer, anti-diabetics, anti-microbial, and blood glucose lowering properties [4, 6], although more scientific research is needed to validate these claims.

Despite achi significant culinary and nutritional values, concerns have arisen regarding the potential contamination of achi seeds by fungal isolates [7, 8]. It is worth noting that the contamination of ach seeds by fungal species may occur during pre-and/or post-harvest periods of the seed, that is, from field to plates. According to [8], food processing which play a crucial role in the preservation and distribution of food can also have significant implications on the tainting of food by fungal species. Key factors that contribute to this are the mishandling of food by sellers, processing equipment, and the increased moisture content of the achi seeds after steeping [2, 8]. High moisture content creates conducive environments for proliferation, spore germination, hyphal development, sporulation, and subsequent mycotoxin production in toxigenic fungal isolates [9].

Unfortunately, the contamination of ach seeds by toxigenic fungal species poses a significant risk to human health. Mycotoxins are harmful secondary metabolites produced by specific fungi, mainly from the *Aspergillus, Penicillium*, and *Fusarium* genera [10, 11]. Mycotoxins of major concern include aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) produced by *Aspergillus* species; ochratoxins (OTA and OTB) biosynthesized by *Penicillium* and *Aspergillus* species; and deoxynivalenol (DON), trichothecenes (T-2 and HT-2 toxins), zearalenone (ZEN), and fumonisins (FB_1 and FB_2) formed by *Fusarium* species [10, 12, 13]. Exposure to mycotoxins in humans can lead to various health issues, including kidney toxicity, liver damage, birth defects, cancer, and immune suppression [14–16]. In severe cases, such as with long-term exposure or acute poisoning, it may cause death [17]. Besides their detrimental effects on health, mycotoxins also result in substantial economic losses for many countries, particularly in Sub-Saharan Africa, due to the expenses associated with ensuring food safety [18].

Regarding achi seeds, traditional processing methods often involve steeping, a pre-treatment process that involves soaking the seeds in water or alkaline solution for a specified period. Steeping is known to influence the physical, nutritional, and biochemical characteristics of achi seeds [1, 2], but there is limited understanding of its impact on fungal contamination of the seed. The few studies [1, 2, 7, 19, 20] that have investigated the presence of fungal contamination in achi seeds have relied solely on conventional identification techniques. It is important to know that these methods might not accurately determine the real identity of the fungal population found in the achi seeds. Therefore, this research was designed to identify the fungal species associated with achi seed sold in some selected markets in Lagos state, Nigeria using morphological and molecular approaches. Additionally, the study aimed to evaluate the impact of steeping on fungal contamination and diversity in the achi seeds.

2 Materials and methods

2.1 Materials

The food samples used in this study include de-hulled steeped and un-steeped achi seeds. The instruments used in this analysis, including stainless steel blender, microscope (JEM Jeol 1010, Ltd., Tokyo, Japan), fabricated dehulling machine, and digital weighing balance were acquired from the Confectionery Processing facility situated at the Federal University of Agriculture, Abeokuta, Nigeria.

2.2 Study sites

Lagos State is divided into three districts: Lagos West, Lagos East, and Lagos Central. For this study, three markets were chosen from each district to ensure a comprehensive representation across the state. The selected markets include Oyingbo, Boundary, and Sangotedo from Lagos Central; Mile 12, Pobo, and Ajah from Lagos East; and Igando, Ile Epo, and Oshodi from Lagos West. These markets were specifically selected due to the high concentration of achi vendors and the availability of achi seeds and related products.

2.3 Sample collection and preparation

Two sets of powdered achi seeds, one steeped and one unsteeped, were collected aseptically in sterile plastic containers from each market. This resulted in a total of nine samples for each type of achi. In addition, unsteeped achi seeds were collected as a control sample. Approximately 10 g of each achi samples were transported on ice to the laboratory for microbial analysis. The control samples were divided into two groups: one group was milled without steeping, while the other group was steeped in distilled water for two days before being milled. All samples were analyzed to determine their moisture content and levels of fungal contamination, following established protocols.

2.4 Moisture content determination

The moisture content of the achi was assessed following the procedures outlined in AOAC [21]. In summary, 2 g of each sample was measured and placed into a pre-dried crucible. These samples were then transferred into a moisture extraction oven set at 105 °C for 3 h. After drying, the samples were moved into a desiccator to cool and then reweighed. This process was repeated until a consistent weight was achieved, indicating that the moisture content of the sample had been accurately determined.

$$Moisture(\%) = \frac{W2 - W1}{W2 - W3} x100 \tag{1}$$

where: W3 = Weight of dish + dried sample; W2 = Weight of dish + undried sample; W1 = Initial weight of empty dish.

2.5 Fungal isolation and enumeration

This study followed the fungal isolation and enumeration method outlined by Adelusi et al. [22]. In brief, 1 g of both blended and unblended achi seeds was accurately measured and placed into sterile test tubes containing 9 mL of peptone water. The mixture was then serially diluted to 10^{-5}. Triplicate 1 mL aliquots of each dilution for every sample were plated onto potato dextrose agar (PDA) using the spread plate technique. These plates were subsequently incubated at a temperature of 27 °C for a duration of 5 to 7 days. Following the incubation period, fungal colonies were visually observed and counted using a colony counter (Gallenkamp, UK). The average fungal loads were then calculated and expressed as colony-forming units per gram of sample (CFU/g) in accordance with Eq. 2.

$$CFU/g = \frac{Number\ of\ colonies\ X\ reciprocal\ of\ the\ dilution\ factor}{Plating\ volume\ (1mL)} \tag{2}$$

2.6 Morphological characterization

Macroscopic and microscopic observations were carried out on the cultures. The macroscopic characteristics of the fungal mycelia such as the colour, shape, texture, size, and aerial hyphae were noted [22, 23]. For microscopic characterization, the fungal isolates were identified under the microscope (JEM Jeol 1010, Ltd., Tokyo, Japan X). Briefly, a small portion of the fungal mycelium was placed on a microscope slide, stained with lactophenol cotton blue, covered with a cover slip, and checked under the microscope. The fungal isolates were identified by assessing their colony morphology and spore characteristics, using the taxonomic keys and guidelines provided by Pitt and Hocking [24]. When traditional methods proved insufficient in accurately identifying individual fungal isolates based solely on their morphological characteristics, molecular analysis was employed to determine their identities. The relative density (RD) of the isolated fungal species was evaluated according to Adelusi et al. [22] as follows:

$$RD(\%) = \frac{Number\ of\ isolates\ of\ a\ species}{Total\ number\ of\ fungis\ iolated} X100 \tag{3}$$

2.7 Molecular characterization

Molecular identification of fungal isolates was performed at the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

2.7.1 Deoxyribonucleic Acid (DNA) extraction

The extraction of DNA from fungal cells was conducted in accordance with the Zymo DNA extraction kit protocol. Approximately 50 mg of fungal cells were combined with 200 µL of isotonic buffer (PBS) containing Tween 20. This resultant suspension was subsequently transferred into a ZR BashingBeadTM Lysis Tube (0.1 mm & 0.5 mm). Following this, 750 µL of BashingBeadTM Buffer was added to the tube, and the mixture was subjected to bead beating at maximum speed for 5 min using a bead beater equipped with a 2 mL tube holder assembly. After bead beating, the ZR BashingBeadTM Lysis Tube (0.1 mm & 0.5 mm) was centrifuged in a microcentrifuge at 10,000 g for 1 min. Subsequently, 400 µL of the supernatant was transferred to a Zymo-SpinTM lll-Filter collection tube and centrifuged at 10,000 g for 1 min. Thereafter, 1,200 µL of Genomic Lysis Buffer was added to the filtrate in the collection tube. Approximately 800 µL of the resulting mixture was then transferred to a Zymo-SpinTM llCR Column in a collection tube and centrifuged at 10,000 g for 1 min. The flow-through was discarded, and this step was repeated. Finally, approximately 200 µL of DNA Pre-Wash Buffer was added to the Zymo-SpinTM llCR Column in a new collection tube and centrifuged at 10,000 g for 1 min. The Zymo-SpinTM llCR Column was then moved to a clean 1.5 mL microcentrifuge tube and centrifuged at 10,000 g for 30 s to elute the DNA.

2.7.2 polymerase chain reaction (PCR) analysis

The PCR was carried out using the primer pair ITS1-5′ TCCGTAGGTGAACCTGCGG -3′ and ITS4- TCCTCCGCTTATTGATAT GC -3′. he reaction volume was 20 µl, and the concentration was adjusted from 5 to 1X, comprising 1X Blend Master mix buffer (Solis Biodyne), 200 µM of each deoxynucleoside triphosphates (dNTP), 1.5 mM MgCl2, 2 units of Hot FIREPol DNA polymerase (Solis Biodyne) with proofreading enzyme, 20 pMol of each primer (Jena Bioscience, Germany), sterile distilled water, and 5 µl of the extracted DNA. Thermal cycling was performed using a Techne (3 Prime Model) with an initial denaturation step at 95 °C for 5 min, followed by 30 amplification cycles of 30 s at 95 °C, 1 min at 58 °C, and 1 min 30 s at 72 °C. Afterwards, a final extension step was performed at 72 °C for 10 min. The resulting amplification product was then separated on a 1.5% agarose gel, with electrophoresis carried out at 80 V for 90 min. Following electrophoresis, the DNA bands were visualized using ethidium bromide staining. A 100 bp DNA ladder (Solis Biodyne) was employed as the molecular weight marker for DNA.

2.7.3 Gel electrophoresis

Agarose (2.25 g) was measured and placed in a beaker. Subsequently, 150 mL of 0.5X TAE (Tris acetate EDTA) was added to the contents of the beaker. The mixture was microwaved until fully dissolved, then cooled before adding approximately 50 µL of ethidium bromide. This cooled mixture was poured into a casting tray with 20 wells and left to solidify. Finally, the casting tray was positioned in an electrophoresis gel tank. Approximately 5 µl of ladder, blank and 4 µl of the PCR product were loaded in the wells of the electrophoresis gel tank and set to run at 80 V for 80 min. Following that, the gel was brought out and viewed using a Biobase Photographed and cutting the Electrophoresis Gel UV-Transilluminator 1289*1024 (Model No UVT-02S with Pixel Density 10 Bit. Biobase Biodustry (Shandong) Co., Ltd, China.

2.7.4 DNA sequencing and phylogenetic analysis

The PCR-amplified fragments of fungal isolates were subjected to 18S rRNA sequencing at the Nigerian Institute of Medical Research (NIMR) using the 3130 Genetic Analyzer from Applied Biosystems. The resulting consensus sequences were searched against the GenBank gene sequence database via BLAST (http://www.ncbi.nlm.nih.gov/, accessed on 10 March 2024) to validate the presumed identity of the fungal isolates. Similarity scores exceeding 90% were considered. A dataset was created by retrieving sequences of closely related fungal species from GenBank, which were then aligned

using Muscle. The evolutionary connection between fungal sequences obtained from the achi seed samples and their reference strains was established using the maximum likelihood (ML) method proposed by Tamura and Nei [25]. Bootstrap values, determined from 1000 replications, were used as parameters for constructing the phylogenetic trees [26]. Branches corresponding to partitions that replicated less than 50% of the bootstrap samples were collapsed. These resulting phylogenetic trees confirmed the evolutionary relationship between the isolated fungal species from this study and their counterparts in the GenBank database. All identified fungal isolates were preserved on slants and in Phosphate Buffer Saline (PBS) with Tween-20 for future use. Additionally, newly generated sequences were submitted to GenBank.

2.8 Statistical analysis

The analyses were performed in triplicate, and the fungal load data underwent analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc.). Furthermore, Duncan's multiple range tests were employed to compare the means at a significance level of 0.05 ($p \leq 0.05$).

3 Result and discussion

Contamination of achi seeds by fungal species can occur at various stages of production, processing, storage, and distribution, posing risks to consumer health [7]. Common sources of contamination of achi seeds include improper storage facilities, inadequate cleaning of milling equipment, high moisture content, and mishandling during processing [8]. By addressing potential sources of contamination and implementing preventive measures throughout the production and supply chain, the safety and quality of achi seeds can be ensured, contributing to consumer confidence and satisfaction. The pictures of achi tree, steeped and unsteeped seeds can be found in Fig. 1.

3.1 Effect of steeping on the achi seed moisture content

The moisture content of the steeped and un-steeped achi seeds is presented in Table 1. Overall, the results indicate variability in the moisture content of achi seeds across different markets in Lagos state, with steeping generally increasing the moisture content of the achi seedS More so, there was significant ($p < 0.05$) difference in moisture content for both steeped and un-steeped achi across all the markets. The moisture content of the steeped achi seeds ranged from 37.82—51.64%, with samples from Ajah exhibiting the lowest (37.84%) moisture content and those from Oshodi market showing the highest (51.64%) moisture content. Regarding the un-steeped achi seeds, the moisture contents varied from 8.32% in achi sourced from Mile 12 to 15.73% in achi obtained from Oyingbo market. The dryness of un-steeped seeds contributed to the low moisture content, aligning with previous research findings on achi powder by Ikegwu et al. [27]. The moisture content of ach seeds, like any food product, can significantly influence its susceptibility to contamination and microbial growth. High moisture content in achi seeds creates favorable conditions for microbial growth, including bacteria, molds, and yeast and this was observed in the steeped market samples having higher fungal load than the un-steeped samples as microorganisms require water to proliferate, hence, the presence of moisture can accelerate their growth rate. The findings of this current study align with the research conducted by Emiri and Chukwu [1], which reported a significantly higher moisture content (15.3%) in steeped achi compared to unsteeped achi (13.9%). As affirmed by Perdoncini et al. [9], foods with higher moisture content are more prone to fungal contamination, as increased moisture promotes the growth of fungal species. The noticeable difference in moisture content observed between steeped and



Fig. 1 **A** = achi tree; **B** = undehulled achi seed; **C** = unsteeped achi seed; **D** = steeped achi seed; **E** = achi powder

Table 1 Moisture content (%) of steeped and un-steeped achi seed collected from some selected markets in Lagos state, Nigeria

Market location	Moisture content of Steeped achi	Moisture content of un-steeped achi
Oyingbo	44.19 ± 0.20^{d}	15.73 ± 0.33^{d}
Boundary	38.92 ± 0.33^{b}	8.46 ± 0.06^{a}
Sangotedo	50.56 ± 0.16^{h}	8.94 ± 0.04^{a}
Mile 12	45.69 ± 0.09^{e}	8.32 ± 0.07^{a}
Pobo	42.72 ± 0.99^{c}	10.52 ± 0.15^{b}
Ajah	37.84 ± 0.42^{a}	15.11 ± 1.20^{d}
Igando	48.59 ± 0.16^{g}	9.23 ± 0.17^{a}
Ile Epo	47.44 ± 0.25^{f}	12.22 ± 0.37^{c}
Oshodi	51.64 ± 0.63^{i}	8.40 ± 0.18^{a}
Control	51.13 ± 0.32^{hi}	8.48 ± 0.01^{a}

Mean values with different superscripts within the same column are significantly different ($p < .05$)

unsteeped samples in this study may be attributed to the absorption of moisture during soaking, which could potentially impact fungal contamination [28].

3.2 Fungal loads in steeped and un-steeped achi seeds

In our study, we determined the fungal counts (CFU/g) in steeped and un-steeped achi seeds sold in selected markets in Lagos, Nigeria. Table 2 illustrates the levels of fungal contamination found in both achi samples, along with control samples. It was observed that steeped achi seeds exhibited higher levels of contamination compared to un-steeped samples in all markets except Pobo market. Fungal counts in steeped achi seeds ranged from 4.92×10^3—5.50×10^3 CFU/g, with the highest mean fungal loads recorded in Ajah and Sangotedo samples at 5.47×10^3 and 5.50×10^3 CFU/g, and the lowest counts in Pobo and Igando samples at 4.92×10^3 and 4.99×10^3 CFU/g, respectively. For the un-steeped achi, the mean fungal load varied from 3.39×10^3—5.33×10^3 CFU/g, with Badagry and Pobo samples having the highest fungal loads of 5.10×10^3 and 5.3×10^3 CFU/g, respectively. The lowest level of contamination (3.39×10^3 CFU/g) was observed in both Oyingbo and Mile 12 samples. Lastly, the control samples showed no fungal count. This finding demonstrates a lower value in comparison to the findings by Omorodion and Nwala [2], wherein they reported a fungal load ranging from 7.0×10^6—5.4×10^7 CFU/g in achi seeds sold in markets around the University of Port Harcourt, Nigeria. Similarly, Oranusi et al. [19] reported a mean fungal load of 8.2×10^9 CFU/g in achi seeds sold across various locations in Owerri, Nigeria.

The elevated fungal load found in the steeped achi seeds, as compared to the unsteeped seeds, may be attributed to the increased moisture content in the steeped seeds [9]. The presence of toxigenic fungal species in achi seeds and their products can compromise product quality and enhance susceptibility to mycotoxins at various stages of production

Table 2 Fungi colony counts of steeped and un-steeped achi seeds collected from some selected markets in Lagos state, Nigeria

Market location	Steeped "achi" seeds (CFU/g)	Un-steeped "achi" seed (CFU/g)
Oyingbo	5.21×10^3	3.39×10^3
Boundary	5.36×10^3	5.10×10^3
Sangotedo	5.50×10^3	3.54×10^3
Mile 12	5.25×10^3	3.39×10^3
Pobo	4.92×10^3	5.33×10^3
Ajah	5.47×10^3	4.47×10^3
Igando	4.99×10^3	3.91×10^3
Ile Epo	5.08×10^3	4.30×10^3
Oshodi	5.43×10^3	3.69×10^3
Control	0.00	0.00

*Values are means of duplicate determination *Mean values with different superscripts within the same column are significantly different ($p \leq 0.05$)

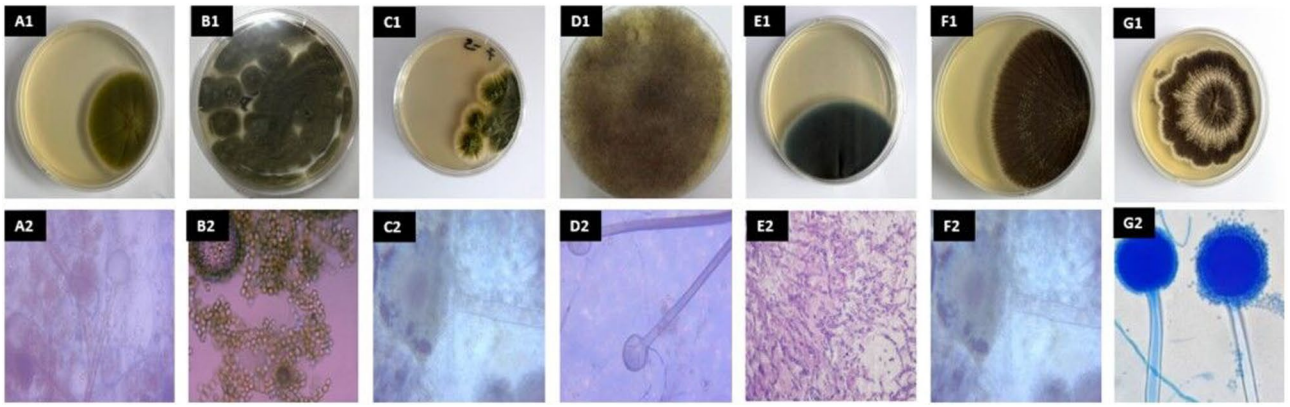


Fig. 2 Colonies and microscopic pictures of some fungal species isolated from steeped and unsteeped achi seeds after day 7 of incubation at 27 °C. A1: *A. oryzae*; A2: microsocpic picture of *A. oryzae*; B1: *A. aculeatus*; B2: microscopic picture of *A. aculeatus*; C1: *A. flavus;* C2: microscopic picture of *A. flavus;* D1: *Rhizopus microspores;* D2*:* microscopic picture *of Rhizopus microspore;* E1*: A. fumigatus;* E2: microscopic picture of *A. fumigatus;* F1: *A. niger;* F2: microscopic picture of A. niger; G1: *A. japonicus;* G2*:* microscopic picture of *A. japonicas*

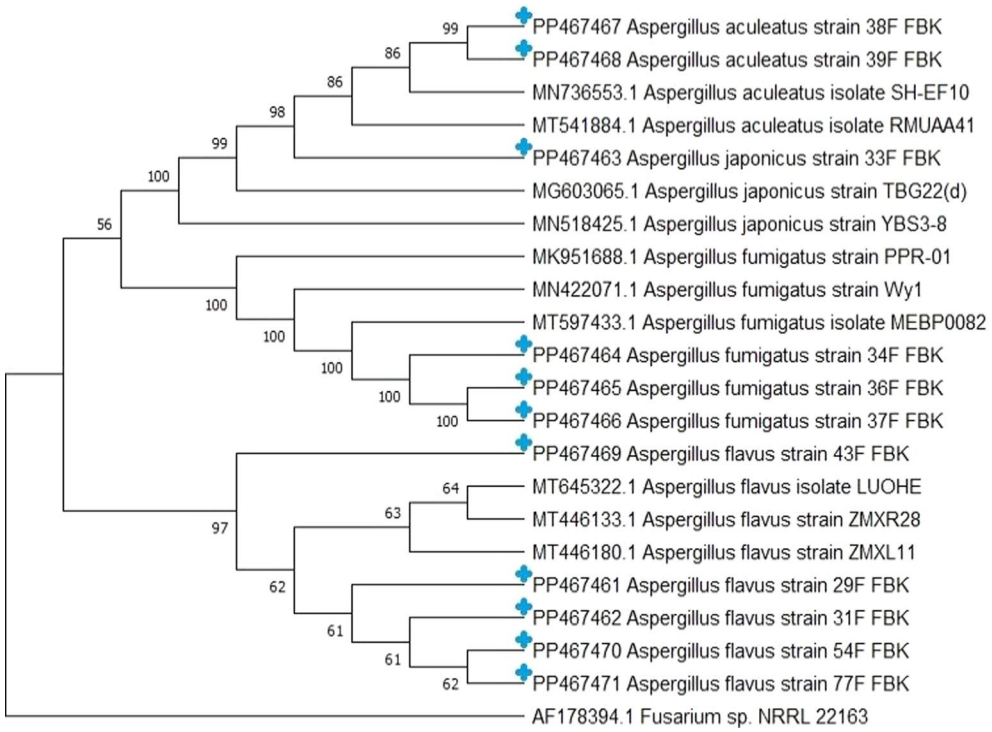


Fig. 3 Phylogeny of *fungal isolates* (taxa names with cross shape) from steeped and un-steeped achi seed sold in Lagos, Nigeria based on ITS-region sequence homology. The numbers depicted within the tree indicate the bootstrap values obtained from 1000 replications. The phylogenetic tree is rooted (outgroup) with *Fusarium* sp

and storage, thereby accelerating deterioration. The contamination of the achi seeds, especially the steeped ones with mycotoxigenic fungi can also be attributed to several factors, such as mishandling of the seeds, including mishandling during steeping, use of contaminated water for steeping, utilization of fungal-contaminated milling machines, and exposure of achi seeds to fungal spores during market display [8, 29].

3.3 Fungal species identification

In this current study, the fungal isolates (Fig. 2) recovered from the achi seeds were identified using both conventional and molecular approaches. Their spores germinated on PDA at 27 °C within a span of 7 days. Colour differences were also observed in the media. Figure 3 represents the phylogenetic tree showing the relationship between some of the fungal isolates. The highlighted strains on the phylogenetic tree are some of the isolated fungal species from the achi samples. Isolates PP467467 and PP467468 were grouped with confirmed *A. aculeatus* (MN736553) with 86% bootstrap

value, while isolate PP467463 was classified with *A. japonicus* strain TBG22. Furthermore, isolates PP467464, PP467465, and PP467466 were grouped with *A. fumigatus* (MT597433) with 100% bootstrap value, whereas PP467461, PP467462, PP467470, and PP467471 were associated with *A. flavus* (MT645322, MT446133, and MT446180). Due to the wide presence of fungi and the complex and ever-changing nature of their taxonomic classification, traditional identification approaches must be complemented with molecular methods to ensure accuracy [23]. The recent examination of phylogeny using 16S rRNA genes unveiled a few new fungal strains in the achi samples. Nevertheless, genetic diversity in fungal species could be attributed to factors such as mutation and recombination [30]. Additionally, the phylogenetic analysis indicated that a majority of the fungal species examined in both steeped and un-steeped achi seeds were closely related to their counterparts in GenBank database.

3.4 Fungi species diversity in steeped and un-steeped achi seeds collected from some selected markets in Lagos state, Nigeria

The data on fungal species diversity in the steeped and un-steeped achi is highlighted in Table 3. The result showed the presence of 7 different fungal species. Interestingly, six of the identified fungal species belong to the genus *Aspergillus*, including, *A. flavus, A. aculeatu, A. fumigatus, A. japonicas, A. oryzae, and A. niger. Additionally, Rhizopus microsporus* was also detected in the achi seeds. Notably, the steeped samples were found to be contaminated with all the seven fungal spp, while the un-steeped achi were contaminated with only four fungal species, namely *A. flavus, A. aculeatus, A. niger*, and *A. fumigatus*. *A. flavus* was discovered in samples from Sangotedo, Mile 12 and Oshodi markets, while *A. niger* were found in samples from both Boundary and Igando markets. Furthermore, *A. fumigatus* was detected in achi samples from Oyingbo, Sangotedo and Ile-Epo markets, whereas samples from Pobo and Ajah markets were contaminated with *A. oryzae* and *A. japonicas,* respectively. Lastly, none of the fungal species were found on the control samples from all the markets. According to Fig. 4, *A. fumigatus* was found to be the most prevalent fungal spccies, accounting for 51.72% of the total fungal isolates in both achi. This was followed by *A. flavus* and *A. aculeatus*, with relative densities of 24.14 and 10.34% respectively. Other fungal species isolated from both steeped and unsteeped achi seeds include *A. niger*, *A. oryzea*, *A. japonicas*, and *Rizophus microspres*, each with a relative density of 3.49%.

Some of the aforementioned fungal species recovered from both steeped and unsteeped achi seed have been reported in previous studies [2, 8, 31]. This current finding is consistent with the findings of Emiri and Chukwu [1], who documented higher rates of *A. niger* (68%), *A. nidulans* (40%), *A. flavus* (30%), and *Rhizopus solonifer* (60%) in boiled achi seeds, compared to the recorded rates of 50%, 38.5%, 15%, and 20% in raw achi seeds, respectively. The authors also observed the presence of *Alternaria alternate* (10%) exclusively in raw achi seeds. Oranusi et al. [19] also confirmed the presence of *Penicillium* sp. in addition to *A. favus* and *A. niger* in achi seeds sourced from various locations in Owerri, Nigeria. According to Ikechi-Nwogu and Chime [7], the prevalent fungal species contaminating achi seeds sold in various markets in River State, Nigeria, were *A. flavus*, *A. niger*, *Botyriodiplodia theobromae*, *Fusarium solani*, *Rhizopus stolonifer*, *P. notatum*, and *F. moniliforme*. In a recent study conducted by Udofa and Ekong [20], *Candida albicans* and *A. niger* were identified as the sole fungal species present in achi seeds collected from markets in Akwa Ibom State, Nigeria. The authors utilized traditional identification techniques, potentially leading to the limited number of identified fungal strains.

Table 3 Fungal species recovered from steeped and un-steeped achi seeds collected from some selected markets in Lagos state, Nigeria

Fungal spp.	Steeped achi seed	Unsteeped achi seed
A. aculeatus	+	+
A. flavus	+	+
A. fumigatus,	+	+
A, japonicas	+	–
A. niger	+	+
A. oryzae	+	–
Rhizopus microspores	+	–
Control	–	–

+ = Presence

– = Absence

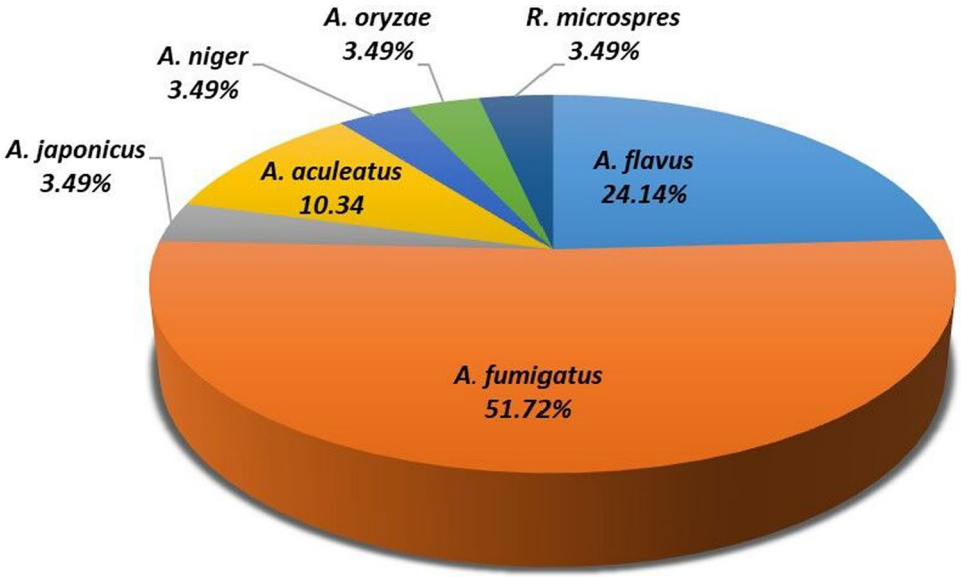


Fig. 4 Percentage occurrence of fungal spp. recovered from steeped and unsteeped achi seeds

Contamination of food items, including achi by various types of fungi poses a significant threat to human and animal health owing to the mycotoxins they produce which are very toxic to humans and animals. Some of the fungal species recovered from the achi seeds are mycotoxigenic in nature. For instance, *A. flavus* is known for the production of aflatoxins, especially AFB_1, AFB_2, AFG_1, and AFG_2 and exposure to high levels of aflatoxins through contaminated food, particularly staple crops like maize and groundnuts, can lead to acute aflatoxicosis, which can cause severe liver damage and even death [14, 17, 32, 33]. Chronic exposure to lower levels is associated with liver cancer, immune suppression, and stunted growth in children [14, 32, 33]. The impact of aflatoxins on food security and public health is particularly severe in regions with inadequate food safety measures, making it a critical issue in many parts of Africa [18]. On the other hand, *A. niger* and *A. aculeatus* are known for the biosynthesis of ochratoxin A [34], which are known to be immunotoxic, tetratogenic, nephrotoxic, and hepatotoxic in humans and animals [35]. Moreover, *A. fumigatus* presents a significant health risk to humans, leading to numerous invasive infections and notable mortality rates, particularly among immunocompromised patients [36]. Research suggests that gliotoxin, a mycotoxin produced by *A. fumigatus*, may also have other harmful effects on humans and animals, such as damage to the genetic material within the cell, lung and liver tissues, and may contribute to the development or progression of liver diseases [37]. Cyclopiazonic acid (CPA), a mycotoxin produced by *A. oryzae*, is not considered a highly potent acute toxin since very high concentrations are required to cause mycotoxicosis in animals and humans. However, CPA intoxication in humans can lead to symptoms such as fever, weight loss, diarrhea, ataxia, and anorexia [38]. Additionally, histopathological effects observed include hyperemia, hemorrhage, and focal ulceration in the alimentary tract, as well as focal necrosis in the pancreas, kidneys, spleen, liver, and heart muscles [38, 39].

4 Conclusion

This study assessed the impact of steeping on fungal load and diversity in steeped and unsteeped achi seeds sold in various markets in Lagos State, Nigeria. The findings demonstrated that steeped achi seeds had higher moisture content, resulting in increased fungal load and greater diversity of fungal species compared to unsteeped achi seeds. The higher moisture content, elevated fungal loads, and greater fungal diversity observed in the steeped achi seeds was attributed to inadequate drying, mishandling, the use of contaminated steeping water, and inappropriate processing methods. The presence of toxigenic fungal species in achi seeds highlights the significance of maintaining low moisture levels in achi seeds. Standardized drying procedures and hygienic practices throughout the production chain are crucial to ensure the safety and quality of achi seeds and their products. Implementing proper hygiene during achi steeping and processing can also help to minimize fungal contamination and mycotoxin production. It is recommended to conduct further research aimed at screening and quantifying different mycotoxins present in the achi seeds. This research would contribute to ensure the safety of achi seeds along their entire production and distribution process, both within the studied areas and worldwide.

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Author contributions Author contributions K.A.O. Conceptualization; Methodology; Data curation; Visualization; Writing–original draft, O.A.A. Visualization; Formal analysis; Investigation; Writing – review and editing, M.I. Investigation; Data curation; Methodology, O.A.M. Conceptualization; Project administration; Methodology; Investigation; Supervision, O.B.O. Project administration; Investigation, Writing–review and editing, O.A.O. Project administration, Investigation; Supervision; Writing–review and editing. All authors reviewed the manuscript.

Data availability The data used in this study will be made available upon request from the corresponding author.

Declarations

Ethics and consent to participate The achi seeds used in this study were purchased from different markets in Lagos State, Nigeria. The collection and use of the achi seeds followed the university's ethical committee guidelines and complied with local regulations governing plant research.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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