Zimbabwe Journal of Science & Technology pp.66-74 Vol.19 [2024]
e-ISSN2409-0360
Zimbabwej.sci.techno

Random coil-rich filamentous fungi contaminates cassava flakes in Ogun State, Nigeria

[1]Benjamin Thoha Thomas, [1]Mercy Olawunmi Coker, [2]Abiodun Noel Thomas, [1]Omolara Dorcas Popoola

[1]Department of Microbiology, [2]Department of Animal Production, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria

Email: benjamin.thomas@oouagoiwoye.edu.ng

ABSTRACT

The role of filamentous fungi in contaminating food grains during the pre- and post-harvest stages is well-documented, and effective control of this agricultural issue may rely on a detailed understanding of their secondary protein structures. This study, therefore, aimed to characterize the secondary structures of filamentous fungi isolated from cassava flakes in Ogun State, Nigeria. A total of 1,000 cassava flake samples (250 from each of the four geopolitical zones in Ogun State) were collected and analyzed for fungal contaminants using standard microbiological and molecular methods. Identified fungal sequences were translated into amino acid sequences and subjected to secondary structure prediction using the SOPMA tool. Results showed that *Aspergillus niger* and *Aspergillus carbonarius* were the most prevalent species ($F = 88.167$, $p < 0.05$), while *Rhizopus stolonifer* was the least frequent, comprising 1.15% of isolates. Secondary structure analysis revealed a predominance of random coil structures across most isolates, except *Aspergillus fumigatus*, which exhibited a higher proportion of alpha helices. Amino acid sequence lengths ranged from 162 to 292 bp. Protein sequence database queries showed no significant similarity to known proteins. Phylogenetic analysis indicated no cluster-specific speciation among the isolates, with species dispersed across various clades. In conclusion, cassava flakes in Ogun State are contaminated with diverse filamentous fungi, predominantly rich in random coil structures, suggesting structural variability that may influence their adaptability and pathogenicity.

Key words: Secondary Structure, Filamentous Fungi, Cassava Flakes

1. INTRODUCTION

Cassava flakes—commonly known as *garri* are the primary form in which cassava is consumed across West Africa, including Nigeria, and indeed throughout much of Africa (Ikediobi *et al*., 1980; Oluwole *et al*., 2004). This cassava food product is a roasted granule of cassava that is widely consumed in both rural and urban areas, either with cold water and/or reconstituted with hot water to form dough, which can be eaten with different types of soup (Oluwole *et al*., 2004). However, certain production and handling practices such as spreading *garri* on the floor or mats to cool, displaying it in open bowls or buckets at market stalls, and using assorted packaging materials to transport the final product from rural to urban areas can significantly increase microbial contamination (Ogiehor and Ikenebomeh, 2005). These microbial contaminants may serve as a vehicle of foodborne diseases (Oyarzabal *et al*., 2003), while filamentous fungi in stored foods can cause discoloration, create-off-odors, degrade nutritional and technological quality, and critically contaminate products with harmful mycotoxins (Basilico *et al.,* 2001; Magnoli *et al.,* 2006; Thomas and Ogunkanmi, 2014).
Filamentous fungi which are central to this study have garnered significant attention because many xerophilic species can produce toxins in agricultural commodities across both temperate and tropical climates. Several studies indicate that mycotoxin biosynthesis genes in most filamentous fungi are regulated by environmental signals rather than being constitutively active,

meaning that changes in factors such as temperature, water availability, pH, oxidative stress, or nutrient levels can trigger or suppress expression of these gene clusters (Peplow *et al.,* 2003; Price *et al.,* 2005). The activation of mycotoxin biosynthetic genes often occurs before the toxins themselves become detectable by conventional analytical methods (Xu *et al.,* 2000; Mayer *et al.,* 2003). As a result, the signaling pathways that trigger mycotoxin biosynthesis during fruit ripening or under poor storage conditions are still not fully understood. To accurately assess whether a food sample may start producing mycotoxins under environmental conditions, ecophysiological studies of these molds are essential. Given the growing importance of preventing mycotoxin contamination at the production stage—rather than attempting to eliminate it afterward, Hazard Analysis and Critical Control Point (HACCP) systems are being enhanced to identify the precise critical control points (CCPs) where mycotoxigenic molds and their toxins can enter the food chain (Aldred *et al*., 2004).

In general, filamentous molds are widespread contaminants of food commodities both before and after harvest (Sanchis and Magan, 2004), including the foods intended for direct consumption without further preparation (Takahashi-Ando *et al.,* 2004; Cavaliere *et al.*, 2006; Trucksess *et al*., 2006). These organisms are especially concerning due to the harm caused to humans and animals by their toxic secondary metabolites called mycotoxins. These mycotoxins are widespread, and several have been classified as Group 2B human carcinogens based on animal studies (Dongo *et al.,* 2008; Jayeola and Oluwadun, 2010). The capacity for mycotoxin production by these fungi depends on multiple factors, including the fungal species involved, composition of the food substrate, and conditions during handling and storage (O'Callaghan *et al.,* 2003). These toxins are commonly found in a wide variety of foods, and their cumulative impacts, such as immune suppression, neurotoxicity, DNA damage, and potential carcinogenicity, are well established (CAST, 2003). Consequently, Ochratoxin A (OTA), which is a type of mycotoxin, has been implicated as a potential risk factor for testicular cancer (Jonsyn-Ellin, 2000). Despite the widespread occurrence of these filamentous fungi in various foods and beverages, there is limited data on the extent of their contamination in cassava flakes (*garri*) samples from Ogun State, Nigeria. Effectively curbing this trend, however, hinges on understanding the secondary structure of these organisms. Accordingly, this study aimed to characterize the secondary structures of filamentous fungi found in Ogun State, Nigeria

2. MATERIALS AND METHODS

2.1 Sources of cassava flakes

A total of 250 samples of cassava flakes were collected from local markets in each of the four geopolitical zones of Ogun State, Nigeria: Yewa, Egba, Remo, and Ijebu, between March 2013 and December 2014. These samples were obtained during both the dry and wet seasons, amounting to a total of 1,000 samples. The sampling followed the statistical guidelines recommended for microbiological testing of foods by the International Commission on Microbiological Specifications for Foods (ICMSF, 2002). Each sample was collected in a pre-sterilized aluminum pan. Any sample whose container lid was opened before reaching the laboratory was discarded. For each sample, details such as appearance, source, and geopolitical zone were recorded. An autoclaved sample of garri was used as a control, while the remaining 1,000 cassava flake samples served as the test group.

2.2 Sample preparation

Ten grams (10 g) of the homogenized laboratory sample were aseptically weighed using a precision digital balance and then transferred into 90 ml of sterile glucose

broth to prepare a 1:10 stock solution. This stock solution was further serially diluted by transferring 1 ml into 9 ml of sterile diluent to achieve a 1:100 dilution. Using sterile pipettes, 1.0 ml of each dilution was added to 9.0 ml of sterile diluent in sterile glass test tubes and mixed thoroughly to create homogenous solutions at each dilution step. This serial dilution process continued progressively until a dilution factor of 10^{-10} was reached.

2.3 Fungal isolation and identification

Aliquots of 0.02 ml from the various serial decimal dilutions prepared earlier were aseptically dispensed in duplicate onto solidified potato dextrose agar using a Pasteur pipette. The inoculated plates were then incubated at 27 °C for five days. Prior to microscopic examination, fungal isolates were subcultured. Wet mount preparations of the isolates were made using lactophenol cotton blue and examined under high-power magnification with dim lighting. Fungi classified under Deuteromycetes (“Fungi Imperfecti”) were identified by their septate and branched hyaline hyphae, following the descriptions provided by Larone (2002). Spores from potential ochratoxigenic fungi were harvested using a sterile inoculating needle into Potato Dextrose Broth and incubated at room temperature for seven days (Larone, 2002).

2.4 Molecular characterization of filament of Filamentous fungi

Each filamentous fungus was suspended directly in 200 mL of sterile saline, and DNA was extracted using a QIAamp DNA Mini Kit (Qiagen). Briefly, samples were pre-incubated at 99 ºC for 20 minutes before following the manufacturer’s protocol. After adding the cell lysis buffer, samples were incubated again at 99 ºC for 10 minutes. The extracted DNA was then amplified by PCR using universal fungal primers (V9D: 5'-TTAAGTCCCTGCCCTTTGTA-3'; LS266: 5'-GCATTCCCAAACAACTCGACTC-3'), targeting conserved regions of fungal rDNA (Pryce et al., 2003). PCR reactions were carried out in 0.2 ml tubes with a total volume of 50 µL, containing 2–10 ng of DNA, 1.5 µL Platinum Taq DNA polymerase (Invitrogen), 200 µM each of dATP, dGTP, and dCTP, 400 µM dTTP, 20 mM Tris-HCl (pH 8.4), 50 mM MgCl2, 0.4 µM of each primer, and 1 µL uracil-N-glycosylase. The amplification protocol included an initial 5-minute incubation at 50 ºC for uracil-N-glycosylase activity, followed by 5 minutes at 95 ºC for Taq polymerase activation, then 35 cycles of 95 ºC for 30 seconds, 62 ºC for 1 minute, and 72 ºC for 2 minutes, with a final extension at 72 ºC for 5 minutes. The PCR products were visualized on agarose gels, purified, and sequenced using a 310 Auto Genetic Analyzer (Perkin Elmer, Applied Biosystems) with the same primers. To serve as an internal control for extraction and amplification, primers targeting the human β-globin gene were included for each sample. DNA sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) and matched to reference sequences with the highest bit score.

2. Secondary structure and evolutionary relationship analyses

Evolutionary analyses of the sequenced isolates were conducted using MEGA Explorer (Tamura et al., 2007), with pairwise distances calculated based on the Kimura 2-parameter model. Within MEGA Explorer, the translation function was applied to convert each gene sequence into its corresponding amino acid sequence, which was then used to perform sequence similarity searches with BLASTP. The best homologous proteins were identified through multiple sequence alignment. The secondary structure of the protein sequences was predicted using the SOPMA tool (Geourjon and Deleage, 1995). The evolutionary relationships of the isolates were inferred using the Maximum Likelihood method under the Tamura-Nei model (Tamura and Nei, 1993). The phylogenetic tree with the highest log likelihood score (-

8931.65) was presented, with bootstrap percentages displayed next to the branches to indicate the frequency of taxa clustering. Initial trees for the heuristic search were generated automatically by applying the Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated via the Tamura-Nei model. The tree topology with the best log likelihood was then selected. This analysis included 10 nucleotide sequences, covering codon positions 1st, 2nd, 3rd, and noncoding regions, with a total of 1,779 positions in the final dataset.

2.6. Statistical Analysis

Frequency distribution was used for calculating the prevalence of the isolated fungal isolates from cassava flakes in the four geopolitical zones of Ogun State, Nigeria. Frequency was calculated by dividing the number of isolated organisms by the total number of fungal isolates and then multiplying the result by 100.

3 RESULTS

3.1 Distribution of filamentous fungi isolated from processed cassava flakes

Figure 1 illustrates the distribution of filamentous fungi isolated from cassava flakes across the four geopolitical zones of Ogun State, Nigeria. As indicated, *Aspergillus niger* and *Aspergillus carbonarius* exhibited the highest isolation rate, with 21 isolates accounting for 24% of all filamentous fungi recovered throughout the zones. Statistically, the occurrence of these molds was significantly greater than that of any other isolated fungi ($F = 88.167$, $p < 0.05$). *Penicillium chrysogenum*, *Aspergillus fumigatus*, and *Absidia glauca* were isolated at rates of 11.4%, 9.2%, and 6.9%, respectively, while *Rhizopus stolonifer* was the least frequently isolated, representing only 1.15% of the total isolates. The molecular amplification results of the various filamentous fungi are shown in Plate 1.

3.2. Secondary Structure of the Isolated Filamentous Fungi

Table 1 presents the secondary structure of the isolated filamentous fungi, highlighting the varying proportions of random coils, extended strands, alpha helices, and beta turns. The amino acid sequence lengths ranged from 162 to 292 base pairs. When these protein sequences were compared against protein databases, no significant similarities with other proteins were detected. Prior to translation, the nucleotide sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) against nucleotide databases, revealing a high degree of identity (98–100%) with the 18S ribosomal DNA gene of the respective isolates. Multiple sequence alignment of the fungal protein sequences demonstrated significant homology and evolutionary relationships among the different isolates.

3.3. Phylogenetic relationship among the isolated filamentous fungi

The phylogenetic relationship among the isolated filamentous fungi from cassava flakes in Ogun State, Nigeria is depicted in Figure 2. As shown in this figure, none of the fungal isolates formed a cluster-specific speciation, but instead, they were scattered across the different clusters with varying degrees of relatedness. In general, *Aspergillus carbonarius, Aspergillus wenti, Fusarium oxysporum*, *Trichoderma atroviride, Rhizopus stolonifera*, *Absidia glauca,* and *Aspergillus terreus were* clustered together, but with evolutionary evidence supporting closer relatedness of *Aspergillus carbonarius. Aspergillus wenti and Penicillium chrysogenum* shares 86% relatedness and clustered together with *Aspergillus fumigatus* at 63% levels of relatedness.

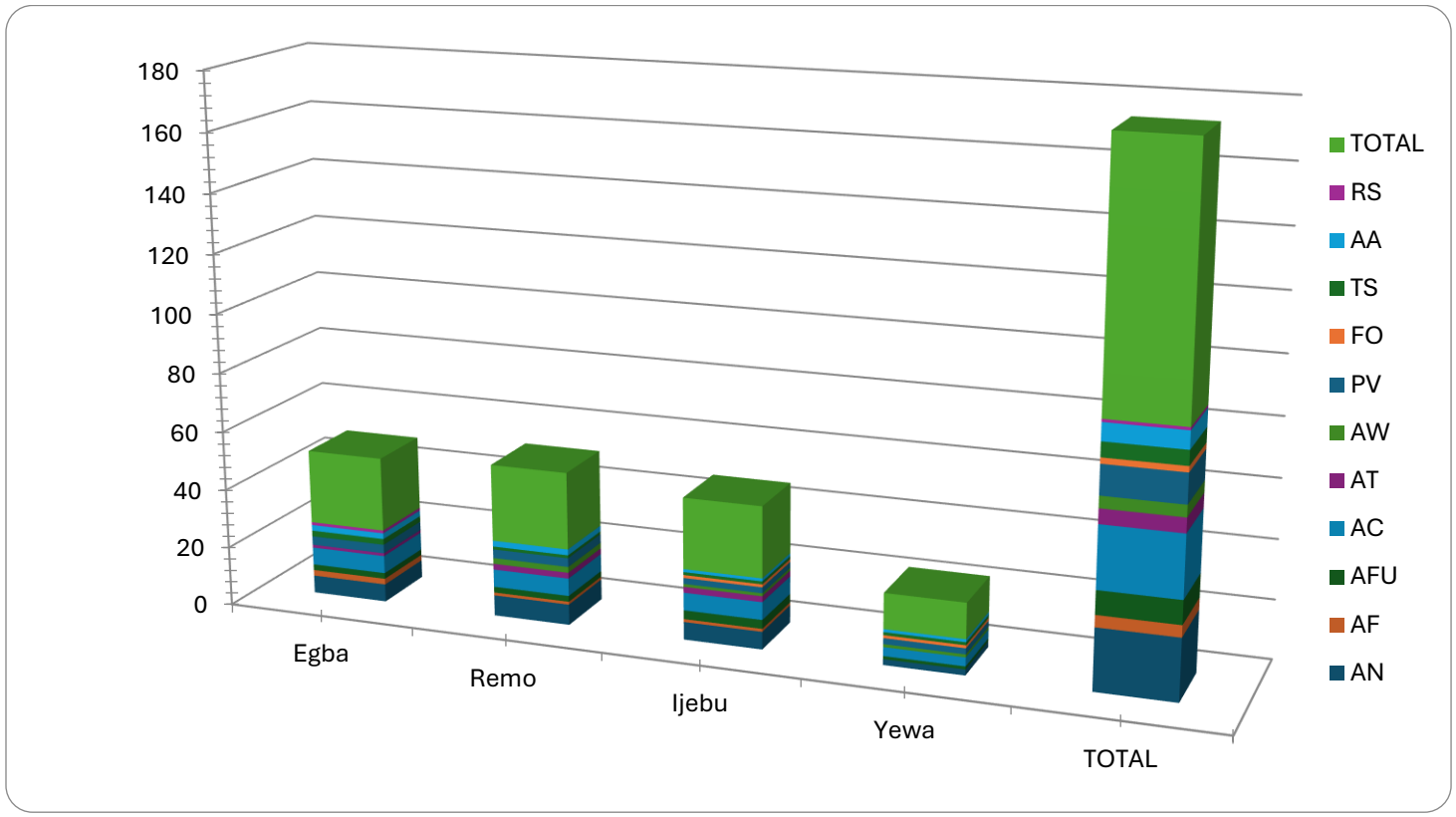

Figure 1: Distribution of filamentous fungi isolated from processed cassava flakes

Key: AC = *Aspergillus carbonarius,* AN= *Aspergillus niger,* AFU= *Aspergillus fumigatus,* AT= *Aspergillus terreus,* AW= *Aspergillus wenti,* PC= *Penicillium chrysogenum,* FO= *Fusarium oxysporum,* TA= *Trichoderma atroviride,* AG= *Absidia glauca,* RS= *Rhizopus stolonifer*

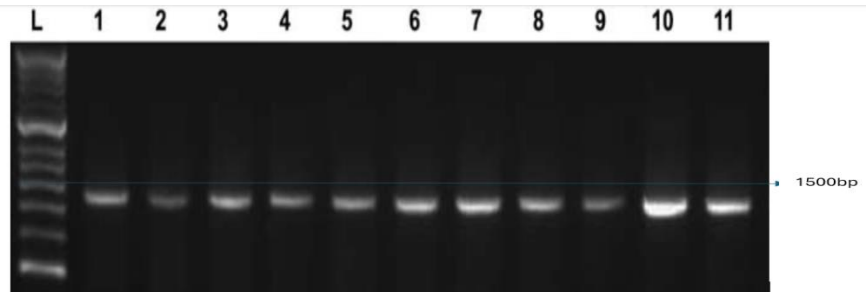

Plate 1: PCR Amplification of some of the Isolated Organisms

Key: 1 = *Aspergillus carbonarius,* 2= *Aspergillus niger,* 3= *Aspergillus fumigatus,* 4= *Aspergillus terreus,* 5= *Aspergillus wenti,* 6= *Penicillium chrysogenum,* 7= *Fusarium oxysporum,* 8= *Trichoderma atroviride,* 9= *Absidia glauca,* 10= *Rhizopus stolonifer,* 11=*Aspergillus flavus.*

Table 1: Secondary Structure of the Isolated Filamentous Fungi

SOPMA PARAMETERS	Occurrence of secondary structure in the sequenced fungal isolates									
	AC	**AN**	**AFU**	**AT**	**AW**	**PC**	**FO**	**TA**	**AG**	**RS**
Alpha helix	70(23.8)	83(29.23)	85(40.5)	40(21)	29(16.38)	32(17.02)	42(25.93)	42(25.9)	28(13.86)	27(11.74)
Extended strand	32(10.96)	65(22.89)	45(21.43)	31(16.3)	37(20.90)	29(15.43)	43(26.54)	43(26.54)	65(32.15)	61(26.52)
Beta	24(8.22)	14(4.93)	14(6.67)	10(5.26)	12(6.78)	19(10.11)	15(9.26)	15(9.26)	8(8.91)	32(13.91)
Random coil	166(56.85)	122(42.96)	66(31.43)	109(57.4)	99(55.93)	108(57.45)	62(38.27)	62(38.27)	91(45.05)	110(47.83)

Key: AC = *Aspergillus carbonarius,* AN= *Aspergillus niger,* AFU= *Aspergillus fumigatus,* AT= *Aspergillus terreus,* AW=*Aspergillus wenti,* PC= *Penicillium chrysogenum,*FO= *Fusarium oxysporum,* TA= *Trichoderma atroviride,* AG= *Absidia glauca,* RS= *Rhizopus stolonifer*

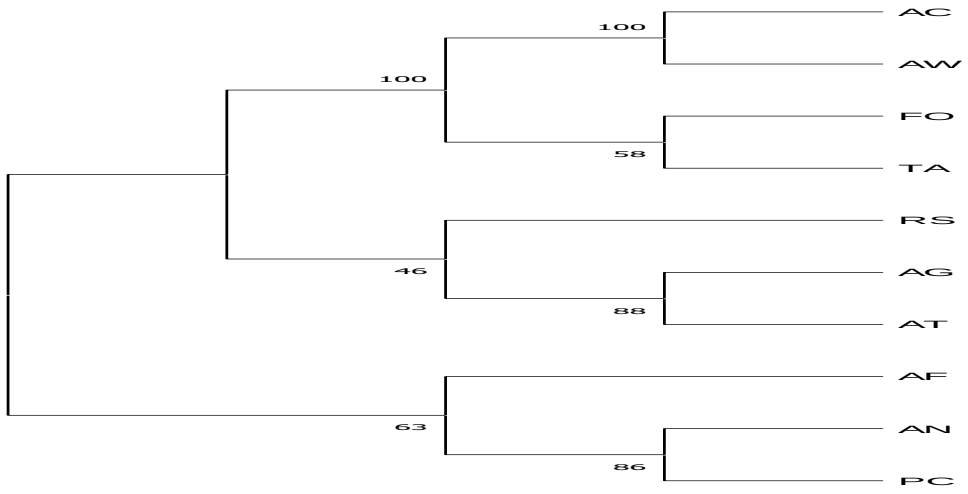

Figure 2: Phylogenetic relationship among the isolated filamentous fungi

Key: AC = *Aspergillus carbonarius,* AN= *Aspergillus niger,* AFU= *Aspergillus fumigatus,* AT= *Aspergillus terreus,* AW= *Aspergillus wenti,* PC= *Penicillium chrysogenum,* FO= *Fusarium oxysporum,* TA= *Trichoderma atroviride,* AG= *Absidia glauca,* RS= *Rhizopus stolonifer*

wenti than that existing between *Fusarium oxysporum* and *Trichoderma atroviride.* Consequently, *Aspergillus niger,* and *Penicillium chrysogenum* shares 86% relatedness and clustered together with *Aspergillus fumigatus* at 63% levels of relatedness.

4. DISCUSSION AND CONCLUSION

The role of filamentous fungi in the spoilage of food products has been extensively documented (Schawn and Wheals, 2004; Ogiehor and Ikenebomeh, 2005; Thomas *et al.,* 2012). In this study, *Aspergillus niger* and *Aspergillus carbonarius* were the most frequently isolated filamentous fungi. This finding aligns with previous reports, as the genus *Aspergillus*, is recognized as one of the most widespread and abundant groups of organisms on Earth (Bennett and Klich, 2003). The predominance of these organisms in cassava flakes may be linked to local production practices in Ogun State, such as drying the flakes on floors or mats, displaying them openly in bowls at markets, and using a variety of packaging materials to transport the finished products from rural to urban areas (Ogiehor and Ikenebomeh, 2005). Some of the filamentous fungi isolated in this study especially *Absidia glauca*, *Trichoderma atroviride*, and *Penicillium chrysogenum*are not known to be commonly isolated from cassava flakes. This discrepancy may be attributed to the larger sample size and the wider range of markets included in this study. Many of the filamentous fungi identified here are known to significantly impact the sensory qualities, microbiological safety, and nutritional value of cassava flakes, thereby contributing to food spoilage (Ogiehor and Ikenebomeh, 2005; Magnoli *et al.,* 2006; Thomas and Ogunkanmi, 2014). The persistent presence of these filamentous fungi in food poses a significant risk to consumers, particularly when their harmful secondary metabolites are produced within the food (Zimmerli and Dick, 1996; Otteneder and Majerus, 2000; Patel *et al.,* 2021).

Most of the isolated fungal proteins showed a strong bias towards random coil structures instead of other types like alpha helices, beta turns, or strands. This suggests that targeting these flexible coil regions could be a useful strategy for controlling these fungi in food (Mohan *et al.,* 2022). This is because these coils are key functional areas of the proteins that are known to be flexible, thus making them easily accessible for targeted disruptions, such as binding by antifungal agents or modifying molecules (Niknam *et al.,* 2025). Such strategies, especially with AMPs, offer a promising, durable alternative to traditional fungicides, lowering the risk of resistance (Ali *et al.,* 2025). Consequently, the significant bias of the isolated filamentous fungi to random coil protein structures is an indication that these secondary structures can be utilized as a proteomic marker for deciphering the diversity of these fungal isolates (Sahay *et al.,* 2020; Ahmad *et al.,* 2021; Popoola *et al.,* 2021).

These secondary structures are formed by hydrogen bonds between atoms in the polypeptide backbone, and importantly, these bonds involve only backbone atoms, not the side chains of amino acids (Rehman *et al.,* 2022). Apart from the fact that the secondary structures play an important role in protein structure and folding, they also allow a loop to escape repair (Zhao *et al.,* 2016) and because they provide flexibility and dynamic interfaces in toxin biosynthetic enzymes, these coils facilitate multienzyme complex formation, subcellular localization, and regulatory signaling, which are all essential for efficient mycotoxin production (Mohan *et al.,* 2022). It is thus imperative to state that studies investigating and targeting coil dynamics offer a novel route to modulate or block fungal toxin biosynthesis.

Necessarily, it has also been pointed out that fungal cells that have structurally rigid protein structures enhance resistance to heat, pressure, and enzymatic degradation,

making them very resilient during food processing and may even protect against antifungal agents that target their structures (Buerman *et al.,* 2021). Hence, disrupting these structures is a strategy to improve food safety. In conclusion, this study has demonstrated that the cassava flakes circulating in Ogun State are contaminated by various filamentous fungi, including those with a random coil-rich protein structure. Therefore, this protein structure could be a target for drug development against these organisms.

REFERENCES

Aldred, D., Magan, N., and Olsen, M., 2004. The use of HACCP in the control of mycotoxins: the case of cereals. *Mycotoxins in Food: Detection and Control*, 139-173.

Basilico, J. C., Debasilico, M. Z., Chiericatti, C., and Vinderola, C. G., 2001. Characterization and control of thread mould in cheese. *Letters in Applied Microbiology*, *32*(6): 419-423.

Bennett, J. W., 2003. Klich. M., Mycotoxins. *Clinical Microbiology Reviews*, *16*(3): 497-516.

Cavaliere, C., Foglia, P., Pastorini, E., Samperi, R., and Laganà, A., 2006. Liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin M1 in cow milk: comparison between electrospray and atmospheric pressure photoionization sources. *Journal of Chromatography A*, 1101(1-2): 69-78.

Council for Agricultural Science and Technology (CAST)., 2003. Mycotoxins: Risks in plant, animal and human systems. *Task Force Report*, pp: 139.

Dongo, L., Bandyopadhyay, R., Kumar, M., and Ojiambo, P. S., 2008. Occurrence of ochratoxin A in Nigerian ready for sale cocoa beans. *Agricultural Journal,* 3(1): 4-9.

Geourjon, C., and Deleage, G. 1995. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Bioinformatics*, *11*(6): 681-684.

Ikediobi, C. O., Onyia, G. O. C., and Eluwah, C. E., 1980. A rapid and inexpensive enzymatic assay for total cyanide in cassava (Manihot esculenta Crantz) and cassava products. *Agricultural and Biological Chemistry*, *44*(12): 2803-2809.

International Commission on Microbiological Specifications for Foods (ICMSF)., 2002. Microorganisms in foods 7: Microbiological testing in food safety management (1st ed.). New York: Springer.

Jayeola, C. O., and Oluwadun, A. O., 2010. Mycoflora and nutritional components of cocoa powder samples in Southwest Nigeria. *Africa Journal of Agri Research, 5*: 2694-2698.

Jonsyn-Ellis, F. E., 2001. Seasonal variation in exposure frequency and concentration levels of aflatoxins and ochratoxins in urine samples of boys and girls. *Mycopathologia*, *152*: 35-40.

Larone, D. H. 2002. Medically important fungi. A Guide to Identification. 4th ed. Washington DC: American Society for Microbiology Press.

Magnoli, C., Hallak, C., Astoreca, A., Ponsone, L., Chiacchiera, S., and Dalcero, A. M. 2006. Occurrence of ochratoxin A-producing fungi in commercial corn kernels in Argentina. *Mycopathologia*, *161*: 53-58.

Mayer, Z., Bagnara, A., Färber, P., and Geisen, R. 2003. Quantification of the copy number of nor-1, a gene of the aflatoxin biosynthetic pathway by real-time PCR, and its correlation to the cfu of Aspergillus flavus in foods. *International Journal of Food Microbiology*, *82*(2): 143-151.

Mohan, N. H., Choudhury, M., Ammayappan, L., Pathak, P., Chakraborty, S., Thomas, R., and Sarma, D. K. 2020. Characterization of Secondary Structure of Pig Hair Fiber Using Fourier-Transform Infrared Spectroscopy *Journal of Natural Fibers*, *19*(11):4223–4235.

Ogiehor, I. S., and Ikenebomeh, M. J., 2005. Extension of shelf life of garri by hygienic handling and sodium benzoatetreatment. *African Journal of Biotechnology*, *4*(7): 744-748.

Oluwole, O. B., Olatunji, O. O., and Odunfa, S. A., 2004. A process technology for conversion of dried cassava chips into “Gari”. *Nigerian Food Journal*, *22*(1): 65-77.

Oyarzabal, O. A., Nogueira, M. C., and Gombas, D. E., 2003. Survival of Escherichia coli O157: H7, Listeria monocytogenes, and Salmonella in juice concentrates. *Journal of Food Protection*, *66*(9): 1595-1598.

Patel, H. K., Kalaria, R. K., Kahimani, M. R., Shah, G. S., and Dholakiya, B. Z. 2021. Prevention and control of mycotoxins for food safety and security of human and animal feed. In *Fungi bio-prospects in sustainable agriculture, environment and Nanotechnology* (pp. 315-345). Academic Press.

Peplow, A. W., Meek, I. B., Wiles, M. C., Phillips, T. D., and Beremand, M. N. (2003). Tri16 is required for esterification of position C-8 during trichothecene mycotoxin production by Fusarium sporotrichioides. *Applied and Environmental Microbiology*, *69*(10), 5935-5940.

Price, M. S., Conners, S. B., Tachdjian, S., Kelly, R. M., and Payne, G. A. (2005). Aflatoxin conducive and non-conducive growth conditions reveal new gene associations with aflatoxin production. *Fungal Genetics and Biology*, *42*(6), 506-518.

Rehman, I., Farooq, M., Botelho. S. (2022).Biochemistry, Secondary Protein Structure. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Retrieved on the 7th June, 2025 @ https://www.ncbi.nlm.nih.gov/books/NBK470235

Sanchis, V., and Magan, N. (2004). Environmental profiles for growth and mycotoxin production. *Mycotoxins in Food: Detection and Control*, 174-189.

Schwan, R. F., and Wheals, A. E.., 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Critical Reviews in Food Science and Nutrition*, *44*(4): 205-221.

Takahashi-Ando, N., Ohsato, S., Shibata, T., Hamamoto, H., Yamaguchi, I., and Kimura, M, 2004. Metabolism of zearalenone by genetically modified organisms expressing the detoxification gene from *Clonostachys rosea*. *Applied and Environmental Microbiology*, *70*(6): 3239-3245.

Tamura, K., and Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and Evolution*, *10*(3): 512-526.

Tamura K, Dudley J, Nei M, Kumar S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24:1596–1599

Thomas, B. T., Effedua, H. I., Agu, G., Musa, O. S., Adeyemi, M. T., Odunsi, O. D., and Oluwadun, A., 2012. Fungi associated with the deterioration of garri (a traditional fermented cassava product) in Ogun State, Nigeria. *Researcher*, 4(2): 1-5.

Thomas, B. T., and Ogunkanmi, L. A., 2014. Ochratoxin A producing filamentous fungi in garri circulating in Ogun State, Nigeria. *Elixir Biosciences*, *75*: 27788-27794.

Trucksess, M., Weaver, C., Oles, C., D'Ovidio, K., and Rader, J., 2006. Determination of aflatoxins and ochratoxin A in ginseng and other botanical roots by immunoaffinity column cleanup and liquid chromatography with fluorescence detection. *Journal of AOAC international*, *89*(3): 624-630.

Xu, H., Annis, S., Linz, J., & Trail, F., 2000. Infection and colonization of peanut pods by Aspergillus parasiticus and the expression of the aflatoxin biosynthetic gene, nor-1, in infection hyphae. *Physiological and Molecular Plant Pathology*, *56*(5): 185-196.

Zhao, Y., Li, H., Fang, S., Kang, Y., Wu, W., Hao, Y., Li, Z., Bu, D., Sun, N., Zhang, M.Q., and Chen, R., 2016. NONCODE 2016: an informative and valuable data source of long non-coding RNAs. *Nucleic Acids Res.,* 44: D203–D208.

Zimmerli, B., and Dick, R., 1996. Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Additives and Contaminants*, *13*(6): 655-668.