*Journal of Research in Forestry, Wildlife & Environment Vol. 16(3) September, 2024*
***E-mail:** jrfwe2019@gmail.com **;** jfewr@yahoo.com*
***http://www.ajol.info/index.php/jrfwe***


***ISBN: 2141 – 1778***
***Kester et al., 2024***



# MOLECULAR DETECTION OF AFLATOXIGENIC MOULD CONTAMINANTS ISOLATED FROM FRAGMENTED STOCKFISH (*Gadus morhua*) RETAILED IN SANGO-OTA MARKETS, OGUN STATE, NIGERIA

*** Kester, C. T[1]., Odesola K. A[2]., Abiona, A.O[2].**
[1]*Department of Agriculture and Agricultural Technology, College of Food, Agricultural Science and Technology, Bells University of Technology, Ota, 112104 Nigeria*
[2] *Department of Biological Sciences, College of Natural and Applied Sciences*
*Corresponding Author: *tckester@bellsuniversity.edu.ng; christieradeke@gmail.com:* +2348030688435

## ABSTRACT

***The occurrence of aflatoxigenic moulds in fragmented stockfish (Gadus morhua), popularly known in Nigeria as Okporoko, retailed in some selected markets in Ota, Ogun State, was investigated and the isolated moulds were molecularly identified. A total of 45 (15 each of Head, H; Trunk, Tr and Tail, Tl) samples randomly procured from three selected markets were analyzed for total mould count following standard procedures. The isolates were grown on Yeast Extract Sucrose (YES) agar and exposed to Ammonium hydroxide to evaluate the phenotypic aflatoxigenicity. Molecular detection of Aflatoxins genes was carried out using the universal primer for moulds (ITS) and four main primers, targeting ver1, Omt1, Nor1 and aflR. The total mould counts ranged from 1.73±0.92 (Trunk) to 2.86±0.17 log CFU/g (Head). The stockfish samples were contaminated with six moulds namely, Penicillium spp. (26.3%), Aspergillus niger (21.2%), Cladosporium spp. (17.3%), Aspergillus flavus (15.0%), Aspergillus fumigatus (10.5%) and Aspergillus nidulans (9.8%). Twenty-six of the isolates were positive to Ammonium hydroxide vapour test of which the PCR-based method detected the four targeted Aflatoxin genes. The occurrence of aflatoxin-producing moulds in the stockfish samples of this study raises concern for public health safety. This necessitates strict monitoring and regulation of handling of fragmented stockfish sold in Ota markets.***

**Keywords:** Aflatoxin, fragmented stockfish, mould contamination, molecular identification.

***Correct Citation of this Publication***
**Kester, C. T., Odesola K. A., Abiona, A.O. (2024** Molecular detection of aflatoxigenic mould contaminants isolated from fragmented stockfish (*Gadus morhua*) retailed in Sango-Ota Markets, Ogun State, Nigeria. *Journal of Research in Forestry, Wildlife & Environment* Vol. 16(3): 70 - 79

## INTRODUCTION

Stockfish (*Gadus morhua*), a wind-dried white fish mainly made from cod, is a centuries-old commodity traded globally (Kuchelmon., 2019). Highly valued for its rich protein, vitamin B, calcium, iron, and Omega-3 content, it is typically imported from Europe into Nigeria (Emokpae and Omongbale, 2016). In the eastern region, stockfish holds significant cultural and economic importance (Subasinghe 2021). The trade of stockfish contributes to the local economy, providing livelihoods for traders and market vendors. It also constitutes an essential ingredient in many traditional recipes.

Stockfish can be kept for many years if stored in a cool dry place. Humidity can, however, cause its deterioration. The highly dehydrated nature of this commodity brands it with a long storage life characterized by mould and insect infestations (Belton *et al*., 2022). According to Emokpae and Omongbale (2016), studies have indicated that even in the exporting countries, where skilled stockfish specialists ensure quality control, quality defects that are hard to identify throughout the drying process may still be

present in the fish. A typical local market with inadequate storage facility required to maintain the initial quality standard \expected for preservation of the stockfish may end up increasing the occurrence of toxigenic moulds in such products, ultimately increasing the risk of mycotoxin production (Atanda *et al*., 2013).

Mycotoxins are secondary metabolites produced by moulds that can cause disease and death in humans and animals (Omotayo *et al*., 2019). They rarely break down in digestion and can consequently remain in the food chain as they are not easily destroyed by temperature treatments such as cooking and freezing (Karlovsky *et al*., 2016). Most food items, including fish, have been identified to be contaminated with residues of mycotoxins and as such potential consumers are exposed to the attendant hazards (Alshannaq and Yu, 2017), some of which are DNA damage and possibly cancer through ingestion of food products (Ahmed Adam *et al*., 2017). The prominent moulds that produce aflatoxins include *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus tamarii* and *Aspergillus bombycis* (Awuchi *et al*., 2020) and according to Benkerroum, (2020), are usually abundant in warm and humid regions of the world. The peculiarity of the prolonged storage and the handling practices exhibited by the retailers of the stockfish product are perhaps responsible for the substantial fragmentation before getting to the consumers. This investigation focuses on the mycological safety of the fragmented imported stockfish in some selected markets in Ota, Ogun State, Nigeria. Aflatoxin-producing moulds in the fragmented stockfish products were identified on molecular platform.

## MATERIALS AND METHODS

### Study site and sample collection

Dried fragmented (head, trunk and tail) stockfish were randomly purchased from five retailers each in Bells Drive Junction (BJ), Iyana Iyesi (II) and Oju Ore (OO) markets in Ota, Ado Odo Local Government Area of Ogun State (latitude 6.6117° N and longitude 3.058°E) Nigeria, between April-May 2021. Fish samples were kept in sterile polythene bags, sealed, labeled and taken to the Microbiology laboratory of Bells University of Technology, Ota for mycological and molecular analysis.

### Isolation of moulds from fragmented stockfish samples

Each of the stockfish samples was aseptically prepared from 10g of the blended samples and thoroughly mixed in 90 ml distilled water. Ten-fold serial dilutions were subsequently prepared up to $10^{-3}$ Potato dextrose agar, PDA (Hi-media) was prepared and made selective for mould growth using 0.2g/L chloramphenicol (Gil *et al.,* 2009). Using spread plate method of isolation, the agar was aseptically poured into sterile Petri dishes and allowed to solidify. 0.1 ml of each of dilutions $10^{-2}$ and $10^{-3}$ was aseptically inoculated and spread on the dry surface of the prepared solidified PDA plates using sterilized glass spreading rod. The plates were incubated at about $28^0$C for 3 to 5 days. The developed mould colonies were observed for their morphological characteristics using color as well as texture of the different colonies formed (Pitt and Hocking, 2022).

### Ammonium Hydroxide Vapour Test

The identified and purified Aspergillus isolates were inoculated on Yeast Extract Sucrose (YES) agar (2% yeast extract, 15% sucrose and 1.5% agar) as single colonies in the center of the plate. This was done by making a well of 5mm diameter at the center of the plate with a sterile cock borer and 1 or 2 drops of concentrated ammonium hydroxide (Sigma- Aldrich) solution were placed on the inside of the lid. The inoculated dish was inverted over the lid and incubated in the dark at 28 °C for 7 days. A change in color of the culture medium was used to determine the toxicity of the isolates. The undersides of the isolates that produced aflatoxin turned pink to red after ten minutes. The non-toxic isolates, however, had no change in color.

### Molecular identification of aflatoxigenic genes

### DNA Extraction of mould isolates

Following the standard protocols for DNA extraction, the isolated moulds (*Aspergillus* spp.) were sub-cultured on fresh PDA and incubated at 25ºC for three days. A small amount of each

isolate was crushed in a sterile mortar and pestle with a Phosphate buffer solution to lyse of the cell walls. A solution of 95µl of water, 95µl of solid tissue buffer, and 10µl of proteinase K were added and mixed thoroughly with a vortex mixer. This was incubated at $55^0$C for 2 hours and centrifuged to remove insoluble debris. The supernatant was transferred to a plain tube and 400µl of Genomic binding buffer was added to 200µl of the supernatant. The mixture was transferred to a Zymo-spin™ IIC-XL column in a collection tube and centrifuged (≥12000×g) for 1 minute. Collection tube was discarded with the flow-through. To the column in a new collection tube, 400µl DNA Pre-wash buffer was added and centrifuged and the collection tube was emptied. Seven hundred micro litres (700µl) of gDNA wash buffer was added and centrifuged for 1 minute and the collection tube was then emptied. Two hundred microliters (200µl) of gDNA wash buffer was added and centrifuged for 1 minute and the collection tube was discarded with the flow-through. The Zymo-spin™ IIC-XL column was transferred to a 1.5ml micro centrifuge tube and 50µl of elution buffer was used to elute the genomic DNA and was stored at -20ºC (Zymo Research Group, USA). The Positive control (*Aspergillus flavus* ATCC 22546) was included in the PCR reaction.

**Polymerase Chain Reaction (PCR)**

Polymerase chain reaction was carried out first with the ITS primers for standardization of the species as moulds while further aflatoxin presence was established with four aflatoxin markers (Nor-1 gene, Omt-1 gene, Ver-1 gene, AflR-1) gene which were all previously identified to differentiate between aflatoxin/ sterigmatocystin producing strains of *Aspergillus* spp. and other non-aflatoxin/ non-sterigmatocystin producing strains of Aspergillus spp (Singh *et al*., 2017). PCR was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix in 20 µl of a reaction mixture and the reaction concentration was brought down from 5X concentration to 1X concentration containing 1X Blend Master mix buffer Buffer (Solis Biodyne, Estonia), 2.0 mM MgCl2, 200µM of each deoxynucleotide triphosphates (dNTP) (Solis Biodyne, Estonia), 20pMol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne, Estonia), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water were used to make up the reaction mixture. Thermal cycling was conducted in a Pielter thermal cycler 100 (MJ Research) for an initial denaturation of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95°C, 1 minute at 62°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. Using Aflatoxin primers (Scherm *et al*., 2005) for PCR amplification, the amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by Ethidium bromide staining, using 100bp DNA ladder as a DNA molecular weight standard.

**Table 2: Primer sequence of the moulds and different aflatoxin genes used for the study**

| S/N | Names | Primers Sequence | Sizes |
|---|---|---|---|
| 1. | ITS-1 | TCC GTA GGT GAA CCT GCG G | 600bp. |
| | ITS-4 | TCC TCC GCT TAT TGA TAT GC | |
| 2. | *Nor-1* | ACC GCT ACG CCG GCA CTC TCG GCAC-3’ | 400bp. |
| | | *5′-GTT GGC CGC CAG CTT CGA CAC TCC G-3* | |
| 3. | *Omt-1* | 5′-GGC CCG GTT CCT TGG CTC CTA AGC-3′ | |
| | | 5′-CGC CCC AGT GAG ACC CTT CCT CG-3′ | 1232bp. |
| 4. | *Ver-1* | 5′-ATG TCG GAT AAT CAC CGT TTA GAT GGC-3’ | |
| | | 5′-CGA AAA GCG CCA CCA TCC ACC CCA ATG-3’ | 895bp. |
| 5. | *AflR-1* gene | 5′-TAT CTC CCC CCG GGC ATC TCC CGG-3′ | |
| | | 5′-CCG TCA GAC AGC CAC TGG ACA CGG-3′ | 1032bp. |

## RESULTS

### Mould population of the dried stockfish

The study analyzed mould species present in different parts of the dried stockfish (*Gadus morhua*) obtained from three markets in Ota. Results showed that there was no significant difference in the distribution of mould species among the parts (head, trunk, and tail) of the stockfish samples in the three markets ($p>0.05$). However, the head parts had the highest mould loads, with samples obtained from Bells Junction (BJ), Oju-Ore (OO), and Iyana Iyesi (II), markets recording 2.86±0.17, 2.79±0.12, and 2.43±0.33 log CFU/g, respectively. The lowest mould loads were found in samples from the fleshy trunk and tail parts of the stockfish obtained from the Iyana Iyesi (II) market, with 1.73±0.92 and 2.47±0.22 log CFU/g, respectively (Figure 1).

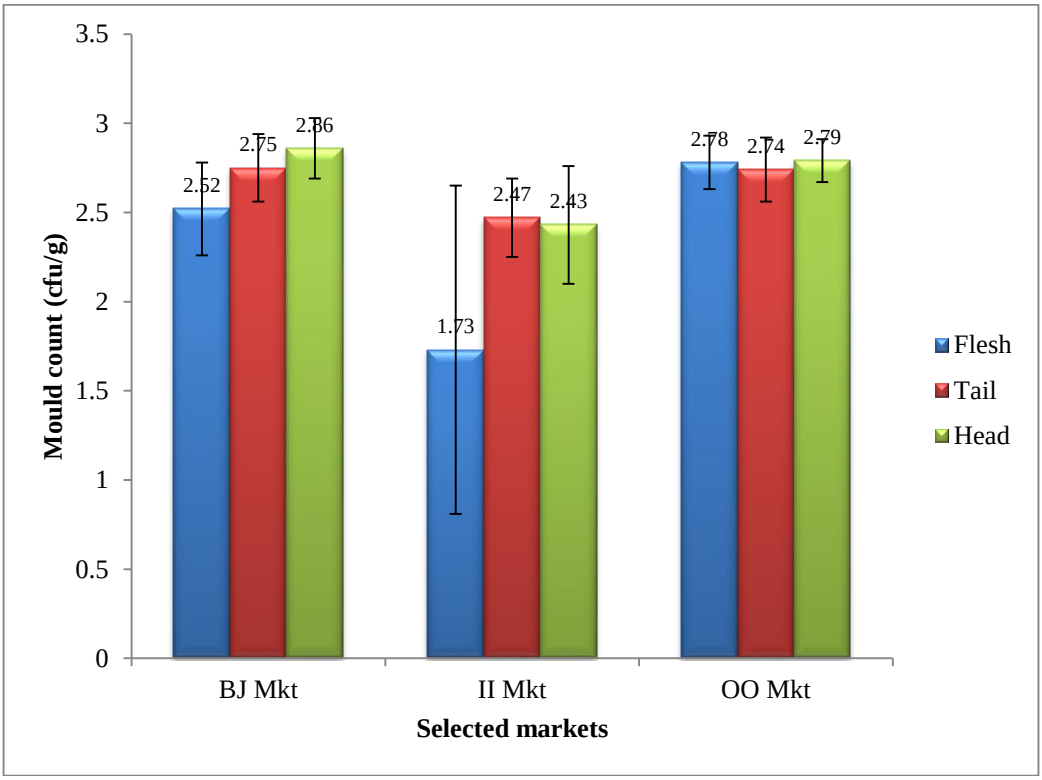


**Figure 1: Mould density in the stockfish parts sampled from the three selected markets**

A total number of 133 mould colonies were recorded as contaminants in the stockfish samples. They included *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Cladosporium* spp. and *Penicillium* spp., and they occurred respectively in a descending order of 26.3% (35), 21.2% (28), 17.3% (23,) 15.0% (20), 10.5% (14) and 13 (9.8%) (Figure 2). The vesicles, spores, and mycelia of the isolated *Aspergillus* spp. are clearly displayed in the micrograph taken at a magnification of X40, (Figure 3).

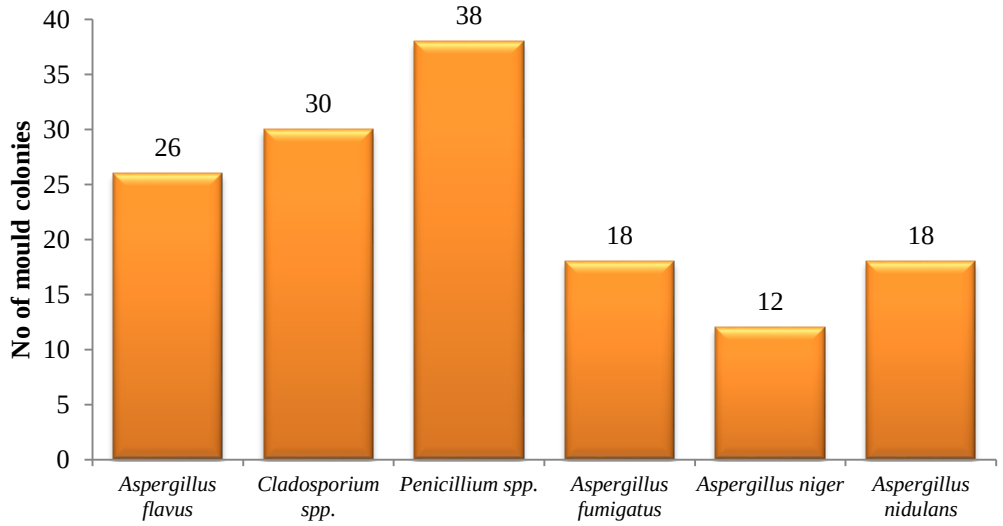


**Figure 2:** Frequency of occurrence of moulds isolated from the fragmented stockfish samples

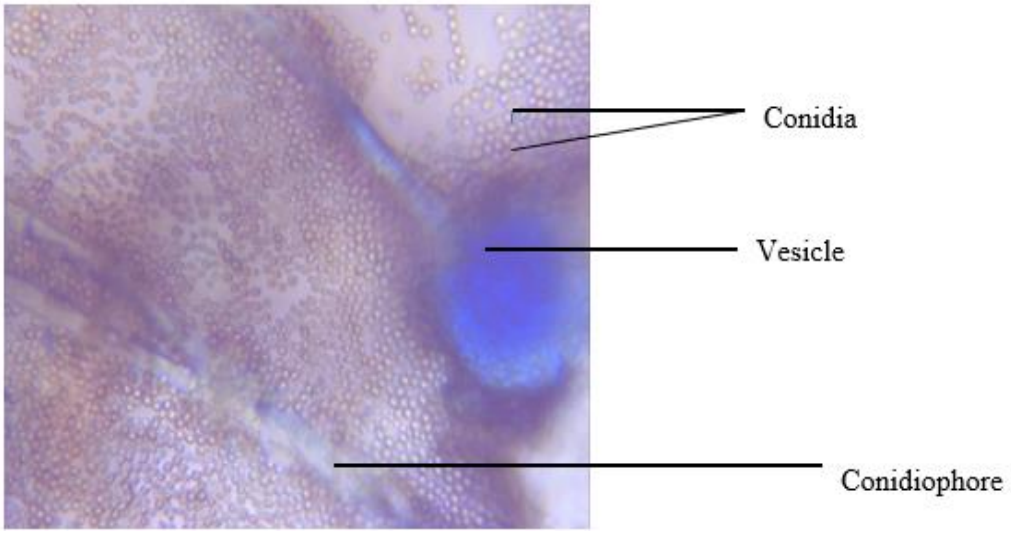


**Figure 3: Micrograph of *Aspergillus* spp.**

**Ammonium hydroxide vapour test for conventional Aspergillus confirmation**

Using the conventional ammonium test to check for the presence of Aspergillus in the 20 isolates identified four out of the 20 *Aspergillus flavus* samples tested. The Aspergillus expresses positive outcomes with a change in colour of the undersides to red within 10 minutes on Yeast Extract (YES) AGAR. In contrast, the remaining 16 samples showed no change in color, indicating that they were non-toxigenic.

**Molecular amplification of the mycotoxigenic gene**

The ITS primers identification shows the PCR detection of the moulds gene amplifying at approximately 600bp with all the 20 samples being positive and detecting the moulds (*Aspergillus sp)* (Plate 1). The four specific primers for the detection of Aspergillus identified the presence of genes involved in the production of Aflatoxin organisms in varied proportion at the expected sizes of 400, 895, 1032 and 1232 bp respectively for *Nor*1, *Ver*-1, *Omt*-1 and *AflR* 1 genes (Plate 2). The markers *Nor1*, *Omt 1 and Ver 1* were significantly informative in picking out the *Aspergillus flavus*, at over 90% while *aflR 1* had just 40% identification of *Aspergillus flavus* in the sample studied.

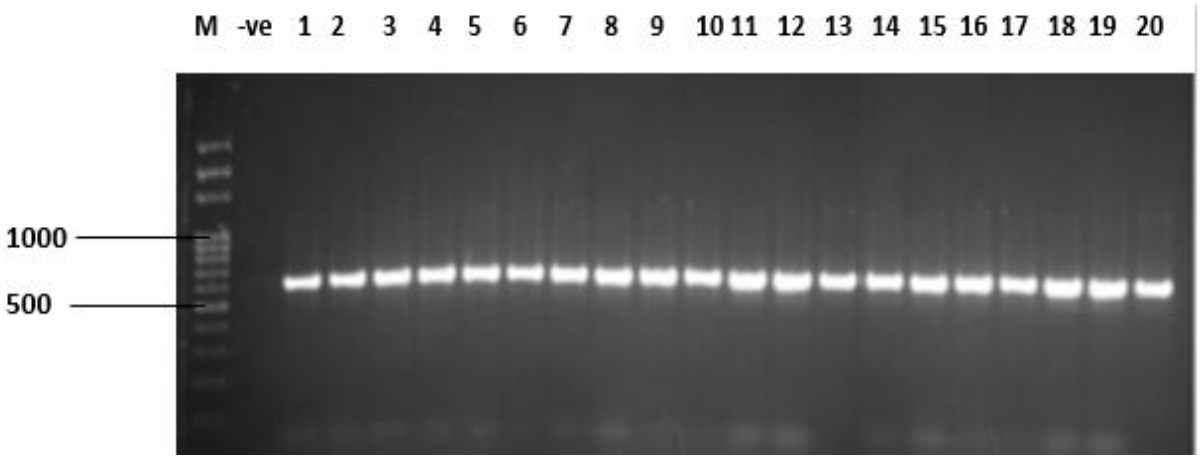


Plate 1: Detection of ITS 1 gene; Lane 1 – DNA Ladder (100bp), Lane 2 - Negative control (without genomic DNA), Lane 3-20 test isolates.

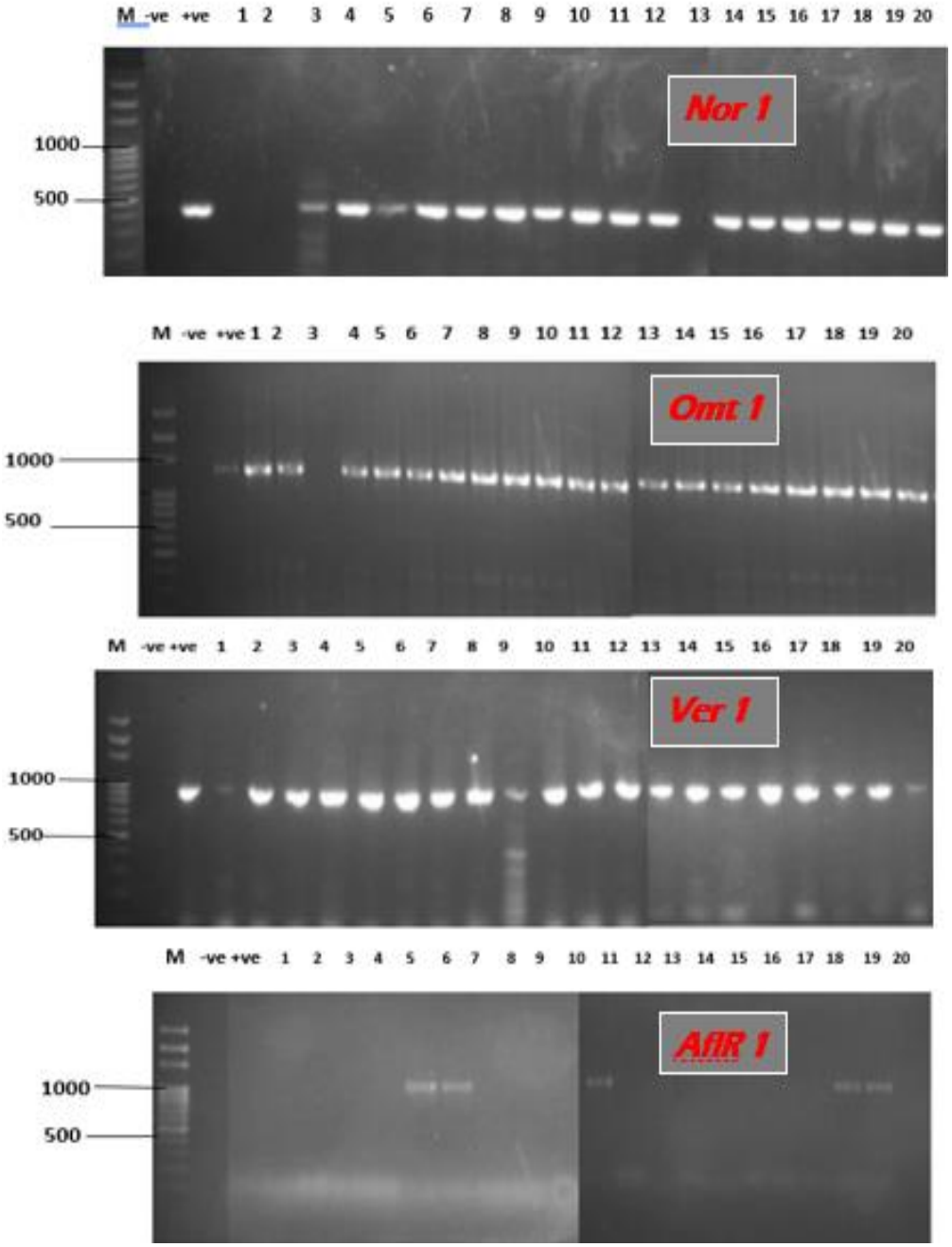


**Plate 2: Detection of *Aspergillus flavus* with *Nor* 1, *Omt* 1, *Ver* 1 and *AflR* 1 genes.**
Lane 1 – DNA Ladder (100bp), Lane 2 - Negative control (without genomic DNA),
Lane 3 – Positive control (*Aspergillus flavus* ATCC 22546) Lane 4-23 were test isolates.

## DISCUSSION

The distribution of the mould load across the three (3) markets showing *Penicillium* spp. *Cladosporium* spp, *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus nidulans* conforms with the report of Junaid *et al.* (2010) in their study of mycotic contamination of stockfish sold in Jos, Nigeria. The spores of these moulds which according to Madueke, *et al*. (2014), naturally disperse from soil through dust in the environment must have settled on the fish products as contaminants. *Aspergillus* spp. has been proven to produce aflatoxin and ochratoxin (Frisvad *et al*., 2019). *Penicillium* spp. is known

to produce aflatoxin and citrinin, toxic mycotoxins that can be harmful to health (Ounleye, and Olaiya, 2015). Vermani *et al*. (2015) reported an allergenic type of *Aspergillus niger* which can induce allergic reactions in some individuals. The occurrence of these moulds in stockfish samples may connote hazard to health, as they can produce toxic metabolites. Cooking stockfish may not eliminate the potency of these harmful metabolites, as mentioned by Sani and Torhile (2016) and they could potentially cause severe damage to the liver and kidneys.

The finding of this study showed mixed growth of moulds in all the stockfish samples obtained from the markets. This occurrence could be attributed to the presence of competitive mycoflora, indicating the co-existence of different mould species associated with stored products (Aboagye-Nuamah *et al*., 2021).

The 26 isolates were subjected to amplification using the universal primers ITS1 and ITS4 for the identification of *Aspergillus* spp. In addition to the ITS primers, four specific primers—Nor1, Ver-1, Omt-1, and AflR1—were used, each targeting key genes involved in aflatoxin biosynthesis. This approach is consistent with the recommendations of the International Aspergillus Working Group, which advocates for molecular identification based on the ITS region for subgenus/section-level classification (Hu et al., 2021). These primers have been widely recognized for their high specificity and sensitivity in detecting aflatoxin-producing strains (Daliri *et al.,* 2023). Nor1 is associated with the early stages of aflatoxin biosynthesis, encoding an enzyme essential for the conversion of norsolorinic acid to averantin. Ver-1 and Omt-1 target later stages in the pathway, which are crucial for the conversion of versicolorin A and sterigmatocystin to aflatoxins B1 and G1, respectively. The AflR1 primer plays a critical role in regulating the expression of the entire aflatoxin biosynthesis pathway (Davari *et al.* 2015),

The high detection rates of Nor1, Ver-1, and Omt-1, each exceeding 90%, highlight their reliability and effectiveness in identifying aflatoxin-producing *Aspergillus flavus* strains. The specificity of these primers makes them indispensable tools for food safety testing and monitoring, where the accurate detection of aflatoxigenic fungi is of paramount importance.

## CONCLUSION

It is revealed from this study that the mould isolates of the stockfish samples of the selected markets were positive to Ammonium hydroxide vapour test of which the PCR-based method detected the four targeted Aflatoxin genes. This implies that the isolated moulds are aflatoxin-producers and their occurrence in the stockfish products could be of public health safety concern for the consuming populace in Ota and its environment. This necessitates strict monitoring and regulation of handling of fragmented stockfish retailed in the markets of Ota and its environment.

### Statement and Declaration

The authors declare no conflict of interest.

## REFERENCES

Aboagye-Nuamah, F., Kwoseh, C.K., and Maier, D.E. (2021). Toxigenic mycoflora, aflatoxin and fumonisin contamination of poultry feeds in Ghana. *Toxicon.* (198): 164-170.

Ahmed Adam, M.A., Tabana, Y.M., Musa, K.B., and Sandai, D.A. (2017). Effects of different mycotoxins on humans, cell genome and their involvement in cancer. *Oncology Reports.* 37(3): 1321-1336.

Alshannaq, A. and Yu, J.H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. *International journal of environmental research and public health,* 14(6): 632 https://doi.org/10.3390/ijerph14060632

Atanda, O., Makun, H.A., Ogara, I.M., Edema, M., Idahor, K.O., Eshiett, M.E., and Oluwabamiwo, B.F. (2013). Mould and mycotoxin contamination of Nigerian foods and feeds. *Mycotoxin and food safety in developing countries*. (68): 1455-1458.

Awuchi, C. G., Amagwula, I. O., Priya, P., Kumar, R., Yezdani, U., and Khan, M.

G. (2020). Aflatoxins in foods and feeds: A review on health implications, detection, and control. *Bull. Environ. Pharmacol. Life Sci, 9*, 149-155.

Belton, B., Johnson, D.S., Thrift, E., Olsen, J., Hossain, M.A.R., and Thilsted, S.H. (2022). Dried fish at the intersection of food science, economy, and culture: A global survey. *Fish and Fisheries*, 23(4): 941-962.

Benkerroum, N. (2020). Aflatoxins: Producing-moulds, structure, health issues and incidence in Southeast Asian and Sub-Saharan African countries. *International Journal of environmental research and public health*, *17*(4), 1215.

Chang P.K., Bennett J.W., and Cotty P.J (2002) Association of aflatoxin biosynthesis and sclerotial development in *Aspergillus parasiticus*. *Mycopathologia.* (153): 41–48

Criseo G., Racco C., and Romeo O. (2008). High genetic variability in non-aflatoxigenic *A. flavus* strains by using Quadruplex PCR-based assay. *International Journal of Food Microbiology.* (125): 341–434.

Daliri, A., Shams-Ghahfarokhi, M., and Razzaghi-Abyaneh, M. (2023). Detection of Aflatoxin B1-producing *Aspergillus flavus* strains from pistachio orchards soil in Iran by multiplex polymerase chain reaction method. *Current Medical Mycology*, 9(3), 1.

Davari, E., Mohsenzadeh, M., Mohammadi, G., and Rezaeian-Doloei, R. (2015). Characterization of aflatoxigenic *Aspergillus flavus* and *A. parasiticus* strain isolates from animal feedstuffs in northeastern Iran. *Iranian Journal of Veterinary Research*, 16(2),150.

Emokpae, A.M. and Omongbale, B.E. (2016). Health risk assessments of some toxic metals in stockfish sold in Nigeria. *Journal of Environmental and Occupational Science.* doi: 10.5455/jeos.2016041310494

Frisvad, J.C., Hubka, V., Ezekiel, C.N., Hong, S.B., Novßkovß, A., Chen, A.J., Arzanlou, M., Larsen, T.O., Sklenář, F., Mahakarnchanakul, W., Samson, R.A., and Houbraken, J. (2019). Taxonomy of Aspergillus section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. *Studies in Mycology*, 93(1): 1-63.

Geisen, R., 1996. Multiplex polymerase chain reaction for the detection of potential aflatoxin and sterigmatocystin producing moulds. Systematic. *Applied Microbiology.* (19): 388-392.

Gil, S. V., Pastor, S., & March, G. J. (2009). Quantitative isolation of biocontrol agentsTrichoderma spp., Gliocladium spp. and actinomycetes from soil with culture media. *Microbiological Research, 164*(2), 196-205.

Hu Z-Y, Liu S-C, Xu Z-G, Liu S-Q, Li T-T, Yu S-L, Zhao W-P (2021). Comparison of *Aspergillus chevalieri* and related species in dark tea at different aspects: Morphology, enzyme activity and mitochondrial genome. *Journal of Food Processing and Preservation.* (45):e15903

Junaid, S.A., Olarubofin, F. and Olabode, A.O. (2010). Mycotic contamination of stockfish sold in Jos, Nigeria. *Journal of Yeast and Mould Research* 1(7): 136 – 141.

Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., Oswald I.P., Speijers G., Chiodini A., Recker T., and Dussort, P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*. (32): 179-205.

Küchelmann, H.C. (2019). Hanseatic fish trade in the North Atlantic: evidence of fish remains from Hanse cities in Germany. *AmS-Skrifter*, (27): 75-92.

Kumar, S., Shekhar, M., Khan, A.A., and Sharma, P. (2007). A rapid technique for detection of toxigenic and non-toxigenic strains of *Aspergillus flavus* from maize grains. *Indian Phytopathology*, 60(1): 31- 38

Liao, J., He, Z., Xia, Y., Lei, Y., and Liao, B. (2020). A review on biosynthesis and genetic regulation of aflatoxin

production by major Aspergillus moulds. *Oil crop Science*, 5(4), 166-173.

Madueke, S.N., Awe, S. and Jonah, A.I. (2014). Microbiological analysis of street foods along Lokoja-Abuja expressway Lokoja. *American Journal of Research Communication*, 2(1): 196-211.

Omotayo, O.P., Omotayo, A.O., Mwanza, M., and Babalola, O.O. (2019). Prevalence of mycotoxins and their consequences on human health. *Toxicological Research*, (35): 1-7.

Ounleye, A.O. and Olaiya, G.A. (2015). Isolation, identification and mycotoxin production of some mycoflora of dried stockfish (*Gadus morhua*). *Academic Journal of Science.* 4(1): 345-363.

Pitt, J.I., and Hocking, A.D. (2022). Ecology of mould food spoilage. In Moulds and food spoilage Cham: *Springer International Publishing*. pp. 3-12.

Sani, F. M., Nasir, I.A., and Torhile, G. (2016). Mycological evaluation of smoked-dried fish sold at Maiduguri Metropolis, Nigeria: preliminary findings and potential health implications. *European Journal of Health Sciences.* (2): 5-10.

Scherm B, Palomba M, Serra D, Marcello A, Migheli Q (2005). Detection of transcripts of the aflatoxin genes aflD, aflO, and aflP by reverse transcription polymerase chain reaction allows differentiation of aflatoxin-producing and nonproducing isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. *International Journal of Food Microbiology.* (98): 201–221. doi: 10.1016/j.ijfoodmicro.2004.06.004. [PubMed] [CrossRef] [Google Scholar]

Singh, R., Rai, T.S., Sharma, N.S., Arora, A.K., and Kaur, P. (2017). Evaluation of a Real time polymerase chain reaction assay for the detection of aflatoxin/sterigmatocystin producing strains of *Aspergillus* spp. *Indian Journal of Animal Research*, 51(4): 676-678.

Subasinghe, R., Siriwardena, S.N., Byrd, K., Chan, C.Y., Dizyee, K., Shikuku, K., Tran, N., Adegoke, A., Adeleke, M., Anastasiou, K., Beveridge, M., Bogard, J., Chu, L., Fregene, B.T., Ene-Obong, H., Cheong, K.C., Nukpezah, J., Olagunju, O., Powell, A., Steensma, J., Williams, G., Shelley, C. and Phillips, M. (2021). Nigeria fish futures. Aquaculture in Nigeria: Increasing Income, Diversifying Diets and Empowering Women. Report of the scoping study. Penang, Malaysia: WorldFish. Program Report: (16).

Vermani, M., Kandi-Vijayan, V., and Kumar-Agarwal, M. (2015). Identification of Aspergillus (*A. flavus and A. niger*) allergens and heterogeneity of allergic patients' IgE response. *Iranian Journal of Allergy, Asthma and Immunology*. 361-369.

Yazdani, D., Zainal, A. M., Tan, Y. H., and Kamaruzaman, S. (2010). Evaluation of the detection techniques of toxigenic Aspergillus isolates. *African Journal of Biotechnology*, *9*(45), 7654-7659.