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Comparative microbial analysis of smoked and frozen fish sold at Ihiala market, Anambra state

Abstract

This study aimed to compare the microbial load of smoked and frozen fish available in the Ihiala Main Market (Uzoigwe) in Anambra State, Nigeria. Eight fish samples, consisting of four smoked and four frozen, were collected in a sterile manner and analyzed microbiologically using the spread plate method. Serial dilutions of the samples were prepared at five different concentrations. The chosen dilutions (10^{-2} and 10^{-4}) were inoculated and cultured on Nutrient Agar, MacConkey Agar, and Sabouraud Dextrose Agar to assess the total viable counts of bacteria and fungi. The isolated microorganisms were identified through cultural, morphological, and biochemical tests, which included catalase, oxidase, citrate, Methyl red, and Gram staining. The findings showed that smoked fish had higher total viable counts compared to frozen fish, suggesting increased microbial contamination. For Nutrient agar (10^{-2}), the values recorded for smoked samples 1 to 4 were 40, 20, 35, 23, and 37. The frozen samples 1 through 4 were measured at 40, 35, 37, and 30. In the SDA (10^{-2}), samples 1-4 of the smoked category were labeled as 5, 2, 3, and 7. For the frozen samples 1-4, the labels were 2, 3, 4, and 1 respectively. The main types of bacteria identified were Escherichia coli, Staphylococcus aureus, Bacillus species, and Pseudomonas species. "Fungal isolates like Aspergillus species." and species of Penicillium. "Were found in certain samples." The existence of these possible pathogens indicates inadequate hygiene during handling, processing, and storage. The study finds that while smoking and freezing can limit the growth of microbes, poor sanitation and contamination after processing can negatively affect the quality and safety of fish. It suggests that there should be stricter compliance with hygiene standards, proper storage practices, and increased awareness among processors and vendors to protect consumer health and improve food safety in Nigeria.

Keywords: Smoked, frozen, fish, dilution, contamination, Aspergillus, penicilum

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Introduction

Fish is an important component of the human diet, and therefore, a large industry operates to produce various types of fish. These products consist of whole fish, both large and small, as well as fish pieces such as cuts and fillets. They also include canned fish in various types, dried and cured items, fish oils and extracts, frozen portions, and complete meals. Each of these variations and their combinations offer many possible results, opportunities, and challenges.[1] Fish nutrition plays a crucial role in various aspects, including color and appearance, which directly influence the quality of the fish. Consumers prefer fish when it is fresh or lightly processed. Ensuring proper manufacturing practices is essential for maintaining hygiene, as the tissues of meat and fish provide an ideal environment for bacteria to grow. Most contamination occurs after shipping, slaughtering, and processing. In a healthy, living animal, the muscle tissue meant for animal food is nearly free of contamination.

Frozen fish is generally regarded as having a lower risk of spoilage and certain pathogens compared to fresh fish, as freezing inhibits microbial growth. However, freezing does not eliminate all microorganisms, and some psychotropic bacteria, spoilage organisms, and certain pathogens or their toxins may survive the freezing process and become active again after thawing.[2] Recent reviews from 2024 highlight that the presence of pathogens, antimicrobial resistance, and changes in the microbiome during processing and storage are significant factors to consider for food safety. Maintaining proper hygiene during the harvesting and processing stages will help reduce the number of microorganisms.

Fish is a significant source of animal protein for people worldwide and is recognized as a beneficial protein source that contributes to overall health. Dry fish made up 25% of the total fish available in the market. Salting and drying is a traditional preservation technique used worldwide, commonly referred to as "salt curing." It is a straightforward, traditional, and cost-effective method for preserving fish. Coastal rural areas are adopting this approach, particularly by utilizing inexpensive fish. Additionally, it is a less expensive food option for low-income individuals in our country and in other nearby developing countries.[3]

Dried fish quality depends on organoleptic, microbiological, and biochemical quality conditions.[4] Being a highly perishable product, fish to be preserved immediately.[4] In India, sun drying and salt drying are considered as the best option for low-valued fishes in 2013-2014, and dry fish export contributed up to 7.86% (819 cores) of all forms of fish exports. The dried fishes retain higher quality standards when compared to fresh fishes and the nutritional quality of dry fish remains unchain.[5] One of the ways to protect fish is by avoidance of temperature abuse during retail display or home thawing.[6]

Materials and methods

The materials used in this research work include:

Autoclave, Pipettes, Incubator, Microscope, Colony counter, Scalpel: Forceps, Petri dishes Freezer, Vortex mixer, fish samples.

Sample collection

A total of 4 samples each of smoked and frozen fish each were bought from local Ihiala markets popularly called Uzoigwe. They were

aseptically wrapped in a sterilized nylon bag. They were transported to the microbiology laboratory for analysis within 2hrs of purchase.

Sterilization of apparatus

All glassware, equipment, and laboratory work surfaces were cleaned and sterilized prior to use. The glassware and equipment were sterilized using an oven and autoclave. The laboratory work benches and tables were cleaned with cotton wool that had been soaked in ethanol. The microbiological analysis was performed using the spread plate count method to quantify microorganisms in the fish samples.

Preparation of media

The media used are Nutrient agar for the enumeration of total heterotrophic count, Macconkey agar for isolation of enteric bacteria while, SDA (Sabourated Dextrose Agar) is for fungi. All media used were prepared according to the manufacture's instruction.

Sample Preparation

A sterilized laboratory forceps and knife were used to cut the fish open and pieces. They were separately put into beakers of sterilized distilled water. They were allowed to stay for 3 hours. This is to allow the water detach every microbial specie in the sample into the water. This water in the beakers will serve as stock solution for serial dilution.

Preparation of culture

The samples were five-fold serially diluted after maceration under aseptic conditions. After the 5-fold serial dilution, 0.1 ml from the second and fourth dilutions were inoculated in duplicates. The culture plates were labeled according to the dilution factor. The appropriate dilutions (10^{-2} and 10^{-4}) were inoculated in duplicates onto the different agar media. All cultures plates were incubated at 37^{0}C for 24-48hours. The bacteria were inoculated on Nutrient Agar for 24hrs, Macconkey Agar for 24hrs and SDA (Sabourated Dextrose Agar for 48hrs. Colonies on plate were counted and multiplied by the dilution factor.

Count (cfu/ml) = count on plate x dilution factor x the number of dilution.

Identification of the bacterial isolate

The bacteria isolated was characterized and identified based on its cultural, morphological and biochemical characteristics.

Cultural and morphological characterization

This involves the growth requirements as well as appearance of colonies (surface, size, and shape) were noticed.

Gram staining

A small layer of bacteria was placed on a clean, grease-free microscope slide, allowed to air dry, and then fixed by passing the slide through a Bunsen burner flame. The slide was positioned on a staining rack and covered with crystal violet for one minute, then rinsed with sterile distilled water. Next, the slide was treated with Lugol's iodine solution for one minute and rinsed again with sterile distilled water. After that, it was decolorized using ethanol for thirty seconds and rinsed with clean sterile distilled water. Iodine was added as a mordant for 60 seconds. The smear was carefully washed with tap water. A solution of 70% ethanol was used for decolorization for a duration of 30 seconds. It was rinsed with clean, sterile distilled water once more and then allowed to dry. Finally, the slide was stained with safranin for one minute and then rinsed with sterile distilled water. The slide was drained and left to air dry before being examined under a lens microscope with immersion oil and a 100x objective lens.

Methyl red test

It is used to assess the capability of bacteria to generate and sustain consistent acid byproducts from glucose fermentation. The test identifies the creation of acid. The test organism was introduced into glucose phosphate broth in a test tube and incubated at 37°C for two days. After this period, five drops of a 0.4% alcoholic methyl red solution were added and mixed well, and the results were recorded immediately. A positive test results in a bright red color, while a negative test shows a yellow color.

Catalase

The test assesses the organism's ability to produce the enzyme catalase, which breaks down hydrogen peroxide into water and oxygen. It is also used to distinguish between bacteria that produce the enzyme catalase. A sterile wire loop was used to collect a small bacteria colony from the sample, and a drop of catalase reagent (hydrogen peroxide) was added to the slide with a sterile pipette. The presence of catalases was indicated by the formation of bubbles, signifying a positive result with oxygen production occurring within 10 seconds. In contrast, a negative result does not produce any bubbles.

Oxidase test

This test was conducted to find out whether an organism has the cytochrome oxidase enzyme. The test is used to help distinguish between different species of bacteria. A clean, sterile petri dish was prepared with a piece of filter paper, to which 2 to 3 drops of fresh oxidase reagent were added. A sample of test organisms was obtained with a sterile wire loop and applied to the filter paper, resulting in a blue-purple color appearing within a few seconds. This indicates a positive test result, while the lack of color indicates a negative result.

Molecular identification of the isolates

Bacterial genomic DNA extraction

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tubes; 750 microliters of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube were centrifuged at 10,000x*g* for 1 minute.

Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 x*g* for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliter was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000x*g* for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000x*g* for 1 minute followed by the addition of 500 microliter of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000x*g* for 1 minute.

The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, 100 microliter of DNA elution buffer was added to the

column matrix and centrifuged at 10,000x*g* microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction.

DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

16S rRNA amplification

The 16s RRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator.

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0.[7] The bootstrap consensus tree inferred from 500 replicates[8] is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method.[9]

Microscopic examination for fungi using lactophenol cotton blue (LPCB) staining

This was done by Placing a drop of LPCB stain on a clean slide. Then a small portion of fungal growth was picked with a sterile needle. It was Teased gently in the stain, then it was Covered with coverslip and observed under ×10 and ×40 objectives.

Results and discussion

Results

The results obtained were recorded as follows (Table 1, Figures 1&2).

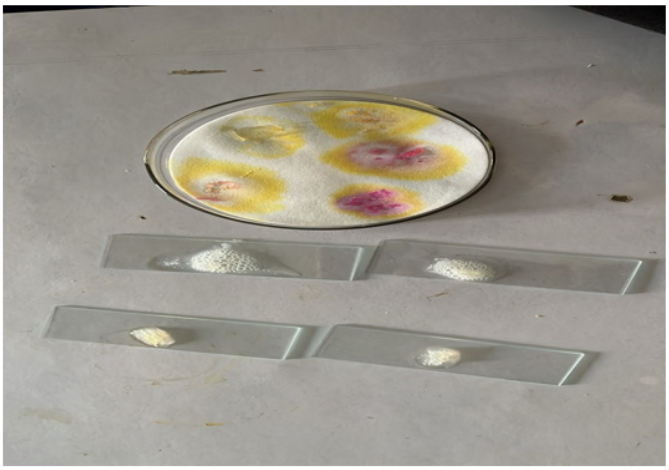

Figure 1 Catalase test and Oxidase test.

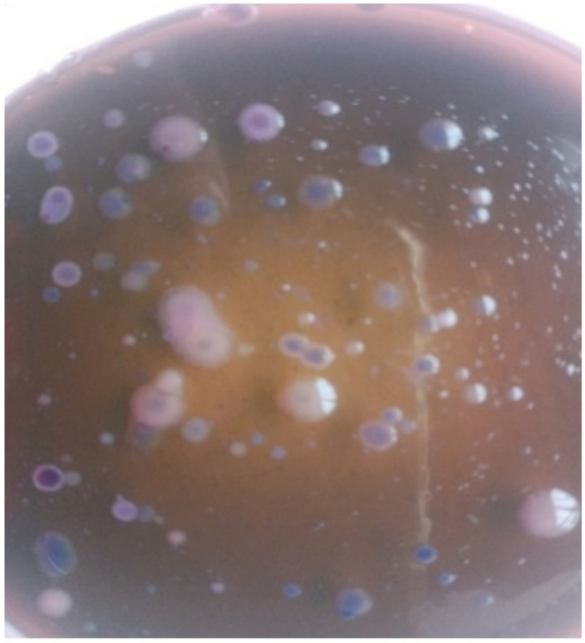

Figure 2 plate of SDA using colony counter.

Table 1 Results of Biochemical Test for Bacterial Isolates from Smoked and Frozen Fish Samples

Possible bacterial isolate	Citrate test	Oxidase test	Gram reaction	Catalase test
Pseudomonas aeruginosa	+ve	+ve	Gram-negative rod	+ve
Escherichia coli	-ve	-ve	Gram-negative rod	+ve
Staphylococcus aureus	-ve	-ve	Gram-positive cocci	+ve
Salmonella spp.	-ve	+ve	Gram-negative rod	+ve
Bacillus spp.	+ve	-ve	Gram-positive rod	+ve

Citation: Ikeokwu OM, Ugochukwu AI. Comparative microbial analysis of smoked and frozen fish sold at Ihiala market, Anambra state. *J Microbiol Exp.* 2025;13(4):139–143. DOI: 10.15406/jmen.2025.13.00447

Result of molecular identification

The obtained 16s rRNA sequence from the isolates produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Salmonella, Escherichia, Staphylococcus* and *Pseudomonas* sp and revealed a closely relatedness to *Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Pseudomonas* aeruginosa (Figures 3-5, Tables 2&3).

Table 2 Result of Fungal Isolates from Smoked and Frozen Fish Samples

Possible fungal isolate	Colour	Presence of hyphae
Aspergillus niger	Black colony	Septate hyphae
Penicillium spp.	Greenish blue	Brush-like conidiophores
Rhizopus spp.	White colony	non-septate hyphae
Mucor spp.	Brownish	-
Candida spp.	Creamy	-

Table 3 Colony counts on the plates after incubation

	Smoked fish								Frozen fish							
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4	
Dilution	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}
Nutrient Agar	40	27	35	23	37	20	30	21	10	3	12	5	12	1	19	4
MacConkey Agar	15	8	13	7	14	9	18	6	3	0	2	0	1	0	3	0
Sabourated Dextrose Agar	5	1	2	0	3	0	7	1	2	1	3	1	4	0	1	0

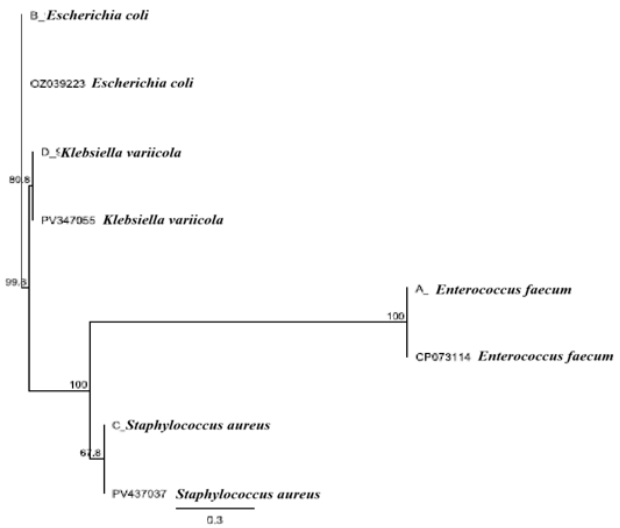

Figure 3 Phylogenic tree of the isolates.

Figure 4 Smoked fish.

Figure 5 Frozen fish.

Discussions

Smoking is an effective preservation method; however, the bacterial spores often remain intact because the smoking process may use insufficient or inappropriate temperatures. As a result, these spores can multiply during storage. Smoking can help manage microbial contamination in fish at sufficiently high temperatures. However, there are instances where high temperatures may not eliminate all types of microbial contaminants, including spores.

The much higher aerobic plate count found in smoked fish suggests a high level of microbial contamination, probably due to handling after smoking, exposure during sales, and inadequate hygiene conditions. Abolagba and Igbinevbo[3] as well as Acharjee et al.[10] have reported similar results in Increased levels of coliform and E. coli. The presence of E. coli in smoked fish, as indicated by counts on MacConkey agar, suggests contamination from feces and inadequate hygiene practices. The presence of Staphylococcus aureus is linked to human handling, highlighting that vendors can be sources of contamination.[11-14]

Frozen fish exhibited lower heterotrophic and coliform counts, confirming freezing as an effective preservation method. However, the presence of psychrotrophic bacteria indicates that freezing does not eliminate microorganisms but only suppresses their growth. Detection of *Salmonella* spp. in both fish types poses a serious public health concern, as these pathogens are associated with foodborne gastroenteritis.[15]

While smoking can eliminate bacteria, other methods may reintroduce bacteria to the fish that is already safe to eat. The practices consist of:

i. While the smoked fish is displayed on the market table, customers periodically approach to inquire about its price. The majority arrive with unclean hands.

ii. Packaging can introduce bacteria to smoked fish that is already safe to eat, even after the smoking process.

iii. Certain fungi and bacterial spores can be present in the air as aerosols.

iv. The majority of vehicles used for transporting fish between markets are inadequate. They can be a source of contamination

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v. Environmental factors, such as air and dust particles, can reintroduce bacteria to smoked fish.

vi. Water used by market women to wash fish can be a source of bacteria.

vii. Infestation caused by fleas and insects that settle on them while being displayed.[16,17]

Suggestion to minimize contamination

I. Fish sellers should use clean and safe water.

II. The smoked fish should be packaged in a clean, transparent nylon bag.

III. Buyers should refrain from handling the fish.

IV. Smoked fish should be displayed in a glass showcase with a frosted light bulb inside to provide warmth.

V. Any unsold smoked fish should be returned to the oven.

VI. Unsold frozen fish should be returned to the cold storage.

VII. The pan displaying smoked fish should be covered to prevent insect infestations.

Conclusion

Because of the above-mentioned factors that can serve as source of contaminants to already smoked fish, this research concludes that,

i. Fish sellers and handlers should observe proper hygiene in their proceeding, packaging and selling of fish.

ii. The study further revealed that many fish sellers face constraints like poor storage/preservation, inadequate capital and poor transportation. Marketing of fish is a profitable enterprise.

Recommendations

This research has revealed that both smoked and frozen fish may nurture various pathogenic microorganisms. The microbial stability of dried fish products depends upon their moisture content. Proper drying procedure is mandatory for achieving a high quality of dried fish. Improve the quality and ensure consumer acceptance it is obvious to run a training session for the dried fish processors and dried fish traders. Other recommendations include

i. Enhanced cleanliness in the processes of smoking, handling, and selling fish.

ii. Implementation of uniform smoking methods.

iii. Ensuring the cold chain is maintained during the storage and distribution of frozen fish.

iv. Regular monitoring of fish products for microbial presence.

v. Education on public health for those who handle fish and for consumers.

Acknowledgements

None.

Conflicts of interest

We declare that there is no conflict of interest of any kind.

References

1. Abila RO. Food Safety in Food Security and Food Trade. *A Case Study: Kenyan Fish Exports*. 2020 Vision for Food, Agriculture and Environment, International Food policy Research Institute, Washington DC. 2003.
2. Adeboyejo AO, Clark OED, Fajana OP. Microbial Load in Five Demersal Marine Fin and Shell Fishes from Nigerian Coastal Waters. *Advances in Oceanography & Marine Biology – AOMB*. 2021;2(4):2021.
3. Abolagba OJ, Igbinevbo EE. Microbial load of smoked fish marketed in Benin City, Nigeria. *Research Journal of Fisheries and Hydrobiology*. 2010;5(2):99–104.
4. Azam K. *Fisherman community of Kuakata, Bangladesh: Fisheries activities and quality of fish*. Bangladesh: Khulna University Balachandran KK. Post–Harvest Technology of Fish and Fish Products. New Delhi, India: Daya Publishing House. Bauer. 2002;77.
5. Arannilewa ST, Salawu SO, Sorungbe AA, et al. Effect of frozen period on thchemical, microbiological and sensory quality of frozen tilapia fish (Sarotherodungaliaenus). *African Journal of Biotechnology.* 2006;18(2):185–192.
6. Holka. Food safety hazards associated with smoked fish. *Journal of Food Safety*. 2024;36(4):1–8.
7. Saitou N, Nei M. The neighbor–joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution.* 1987;4:406–425.
8. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*. 1985;39:783–791.
9. Jukes TH, Cantor CR. *Evolution of protein molecules*. In: Munro HN, editor. *Mammalian Protein Metabolism.* Academic Press, New York. 1969;21–132.
10. Acharjee M, Ahmed E, Munshi SK et al. Validation of γ–irradiation in controlling microorganisms in fish. *Nutrition and Food Science*. 2014;44(3):258–266.
11. Abolude DS, Opanbumi OO, Dan–Davies OA. Fresh Water Fungi Associated with Eggs and Broodstock of African Catfish (Clarias gariepinus Burchell 1822) in Fish Hatchery Farms, Zaria, Kaduna State, Nigeria. *Journal of Research in Environmental Science and Toxicology*. 2013;2(7):131–135.
12. Adebayo–Tayo BC, Odu NN, Anyamele LM, et al. Microbial Quality Of Frozen Fish Sold InUyo Metropolis. Nature and Science. 2012;10(3):71–77.
13. Adebayo–Tayo BC, Adegoke AA, Akinjogunla OJ. Microbial and Physico–Chemical Quality of Powdered Soymilk Samples in Akwa Ibom, South Southern Nigeria. *African Journal of Biotechnology*. 2009;8(13):3066–3071.
14. Adedeji OB. *Principal Human Disease Resulting from Ingestion or Contact with Fish and Shellfish.* A PowerPoint Lecture Supplement in the Department of Veterinary Public Health & Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. 2012.
15. Adesokan HK, Funso–Adu K, Okunlade OA. Foodborne pathogens on meat stored in major central cold rooms in Ibadan, Nigeria. *Int J Animal Vet Adv*. 2020;3(4):249–254.
16. Al–Sheraa AS. Microbial quality of three imported fresh locally produced marine fishes in Al Faw City, Basrah, Iraq. *Journal of Aquaculture Research and Development.* 2014;9(4):531.
17. Atapattu R, Samarajeewa U. Fungi associated with Mycopathologia, dried fish in Sri Lanka. 1990;111(1).