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Isolation and Identification of Fungi Associated with the Spoilage of Smoked Fish

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Abstract

Smoked catfish (Clariasgariepinus) is one of the most popular types, of fish products sold in Bidamarkets, Niger state, Nigeria. A research is conducted on the smoked catfish vended in the markets with the aim of providing firsthand information on the safety issues associated with the consumption of this fish. A total of five (5) samples were collected from (5) different locations in Bida. The samples were analyzed using pour plate method on SabourgaudDextrose Agar (SDA) to determine fungal isolates. The isolates were characterized by macroscopic and microscopic characteristics. The result from this study revealed a highest mean fungal count of 9l.0±5.566CFU/mL from smoked catfish obtained from Old market. The least fungal count of 48.0±11.31CFU/mL was found from the smoked fish obtained from Big gate. The result also revealed the presence of five different fungal species associated with the smoked catfish samples with Aspergillusniger as the most prevalent 30.7%, followed by Aspergillusglaucus 21.2%, Rhizomucorsp 19.2%, Trichophytontansurans 15.3% and Mucorsp were the least abundant fungal species on the smoked catfish accounted for 13.5% each. The present level of fungal contamination of the smoked catfish can probably be either due to the level of moisture in the smoked catfish or unhygienic handling. Attention of the healthcare providers and the authorities concerned is therefore needed towards orienting the retailers and the consumers towards proper methods of storing such smoked fish, and that the consumers have to properly boil the fish before consumption.

Introduction

Fish is highly nutritious with high protein content. However, it is a suitable medium for growth of microorganism, if poorly processed (Oparaku and Mgbenka, 2022). The growth ofmicroorganisms and other non-microbial activities such as lipid oxidation contribute to the deterioration of fish products (Martin, 2020). An increase in the ambient temperature triggers favorable conditions for microorganisms to thrive, which reduces the quality of fish and its potential keeping time leading to food loss (Abolagba*et al*., 2021). Preserving food and other perishable products like fish and meat generally involves processes that impede growth of

microorganisms either by the addition of growth inhibiting ingredients or adjusting storage conditions by freezing or drying (Akise*et al*., 2023). Processing methods affect the microorganisms in fish in different ways, resulting in different types of micro-flora and different risks from spoilage organisms and pathogens. In dried fish, the micro-flora is prevented from growing by the storage method used and the product may have a long shelf life in the preserved state. However, the microbial load of fish rarely indicates the quality of the fish, but gives an indication of the risk of spoilage induced since each of the organisms has different ways of affecting the health conditions of consumers of such contaminated fish (Gram *et al*., 2019). As a result there is a need to investigate microorganisms such as fungi that are associated with fish spoilage. This necessitated this study into investigating fungi associated with smoked dried fish vended in open markets in Bida, Niger State.

The smoking processes of fish are of two forms which are, wet hot smoking and dry hot smoking (Agriculture Nigeria Online Hub). Both processes are carried out at temperatures high enough to cook the fish. Wet hot smoking usually takes about 1 to 2 hours and yields a moist, versatile product with about 40 to 55 percent moisture content, while dry hot smoking, which is usually preceded by the former process, takes about 10-18 hours, sometimes days and yields fish with 10 to 15 percent moisture content. In the tropical countries such asNigeria, (Akande and Tobor, 2019) and Olokor*et al*., (2019) documented that smoke dryingof fish is one of the oldest available local forms of preservation methods essentially employed by most fishing communities. Smoke drying, apart from giving the product desirable taste and odour, preserves and prolongs the shelf-life of fish products conveniently at ambient condition through its anti-bacteria and oxidative effects, lowering of pH, imparting desirable colouration, accelerating the drying process and acting as antagonist to spoilage agents (Sani*et al*., 2020). In Nigeria, fish can be eaten as fresh, preserved or processed (Tobor,2018)reported that the percentage composition of the different processing and retailing methods of fish for consumption in the artisanal sector are as follows live fish 7%,fresh fish 27%,smoke fish 45%, sun dried 11%, and salted and sun dried 10%. Smoke drying methods used in Nigeria requires low capital, investment, and it is conducted in fisherman camps and fish processing centers in traditional smoking kilns of clay, cemónt blocks, drums or iron sheet (Eyo,2020). This has resulted in a very short shelf life and low market value of the fishes as well as inability to withstand handling and transportation by retailers (Akandeand Tobor, 2019). The aim of the study is to investigate the fungi associated with spoilage of smoked catfish.

MATERIALS AND METHOD

Study Area

The study was conducted in Bida, a Local Government Area in Niger State, Nigeria and city on the A124 highway which occupies most of the area. The Local Government Area has an area of 51km^2 and a population of 188,181 at the 2006 census. The postal code of the area is 911.

Sample collection

A total of five (5) samples of smoked dried fish were bought at randomly, from five(5) different markets; New market, Old market, small market, Big gate and Small gate, in Bida town, Niger State, Nigeria. The samples were carefully packed into separately well labeled sterile polythene bags and kept in a clean container and transported to the laboratory of the Department of Biological Sciences, The Federal Polytechnic Bida, Niger State, where they were properly identified according to the identification keys described by (Holden *et al*., 2020) weighed individually and stored in a refrigerator prior to analysis.

Equipment Used

The equipment's used in carrying out the study are as follows;

Test tube

Test tube rack

Conical flask

Electric blender

Autoclave

Cork borer

Microscope

Inoculating loop

Preparation of Culture Media

4.5g of the SDA was measured into 250ml beaker and 150ml distill water was added to the beaker containing the Sabourgaud dextrose agar (SDA). The mixture was shaked to attainhomogenous mixture then made air tight with cotton wool and was autoclaved at 121°C at the pressure of 15psi for 15minutes. The media was poured into 10 Petri dishes labeled respectively. The media was allowed to cool then,lml of serial dilution of spoilt fish sample was introduced to the media in the Petri dishes covered. The cultured Petri dish of the fungi was kept for 72 hours to observe growth.

Isolation of Fungal Flora

One gram of each fish samples was taken and crushed in a sterile mortas with pestle. Nine milliliters sterile water was added and serially diluted up to 10^{-4}fold. Thereby, 1ml from each suspension was poured plated using freshly prepared Sabourgaud'sDetrose Agar. The plates were covered and gently swirled to mix and allowed to gel, the Sabourgaud's Dextrose Agar plates were inverted and incubated at 25°C for 24 hours.

Identification of Fungal Flora

The fungal isolate were identified base on the macroscopic and microscopic characteristics as described by (Fayola and Oslo, 2019), (Pepper and Gerber, 2020) and(James and Natalie 2019). Microscopy was carried out by observing the morphological characteristics like size, shape, growth and color of the plate. Microscopic identification of fungi and fungus like organism involve the observation of morphological features such as shape, size of hyphen, shape of sporangia conidia conidiophores and spores. This Was done Using a flamed inoculating needle, the edge of each colony is picked and slides of the different colonies are made, a drop of lacto phenol cotton blue stain is added to the slides and covered with cover slip and examine under the microscope Using x100magnification. The resultant microscopic characteristics were compared with the scheme provided by (Claus, 2018) and Ellis *et al*., (2019).

RESULT AND DISCUSSION

Results

Table 1 shows the Mean Fungal Count (MFC) of the fungal isolate of smoked catfish collected from 5 different market locations. The highest mean fungal count was recorded in old market at 91.0± 5.66CFU/ml while the lowest mean count was recorded in Big 48.0±11.31 CFU/ml since P.value 0.020<0.05, the test shows that there is significant difference between the mean colony counts of fungi obtained from smoked catfish at 5% level of significant.

Table 2 shows the macroscopic and microscopic characteristics of the fungal isolates obtained from smoked catfish samples. From the results obtain, a total of 5 fungal isolates were identified namely; *Mucorsp*, *Aspergillusglaucus*, *Rhizomucorsp*, *Trichophytontansurans*and*Aspergillusniger*.

Table 3 shows the distribution of fungal isolated from smoked catfish of different market locations. The smoked catfish obtained from Old market shows the highest fungal isolates at 25% follows by New market, Small market, Small gate at 23% each, and the least fungal isolate was obtained from Big gate at 13%.

Table 4 shows the prevalence of fungal isolate from smoked catfish. Five different species were isolated from the spoiled catfish, with *Aspergilusniger* as the most prevalent 30.7%, followed by *Aspergijiusglaucus* 21.2%, *Rhizomucorsp* 19.2%, *Trichophytontansurans*l5.3% and *Mucorsp* were the least abundant fungal species on the smoked catfish accounted for 13.5% each.

Table 1: Mean Colony counts of fungi flora obtain from the Smoked catfish sample

Sample Code	10^{-1}	10^{-2}	Mean±SD	P Value
BG	56	40	48.0±11.31	
NM	86	70	78.9±11.31	0.020
SM	92	84	88.00±5.66	
OM	70	58	64.00±8.49	
SG	95	87	91.00±5.66	

P value is less than 0.05

Chart

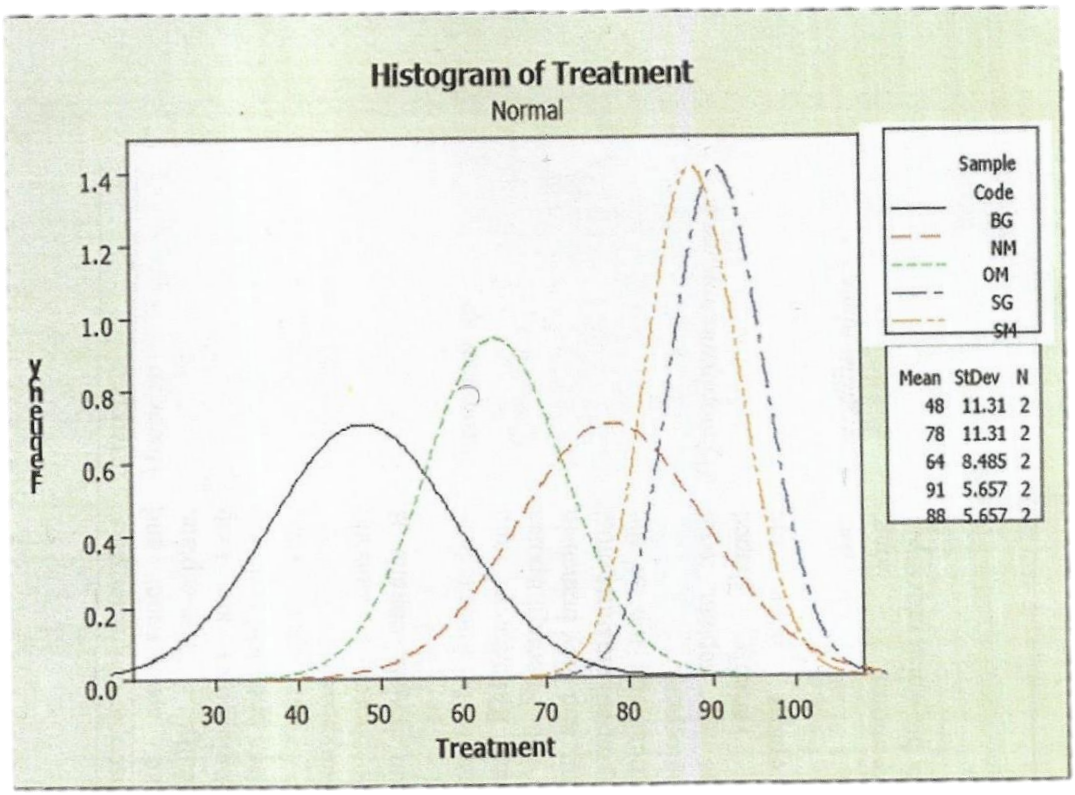

Table 2: Macroscopic and Microscopic Characteristics of fungal isolates from smoked catfish sample

S/NO	Sample	Macroscopic Characteristics	Microscopic Characteristics	Inference
1	BG	White to gray cotton candy growth	Hypae are wide and practically non-septate, sporangiospores are long and often branched and bear terminal round, spore filled.	*Mucorsp*
2	NM	Surface felt like dull green with yellow areas of cleistothecia production. Reverse white to yellow	Septatehyphae., conidiosphores smooth, medium length, conidia is rough	*Aspergillusglaucus*
3	SM	Very fluffy growth with texture of cotton candy, gray becoming dark brownish with age, reverse is white.	Appears to be intermediate between Rhizospores and mucor spp. Sporangiospore are long, and they branch in a sympodial manner, most often near the top of sporangiospore	*Rhizomucorsp*
4	OM	Highly variable, surface maybe white, grayish, yellow, rose, or brownish. Reverse is reddish brown	Hyphae are septate, with many variably shaped microconidia along the hyhae.	*Trichophytontonsurans*
5	SG	Surface is black with white border,thallus is deep visibly composed of long, white cream erect at the apices. Reverse white to cream	Septate hyphae, conifiosphore long, smooth, brownish near top.	*Aspergillusniger*

Table 3: Distribution of fungal isolates amongst different market sites

Sample Code	Number of occurrence	Percentage (%)
BG	3	5.8%
NM	12	23%
SM	12	23%
OM	13	25%
SG	12	23%
Total	52	100%

Table 4: Prevalence of isolated fungal flora

S?NO	Fungal isolates	Frequency	Prevalence
1	*Aspergillusniger*	16	30.7%
2.	*Aspergillusglaucus*	11	21.2%
3.	*Rhizomucorsp*	10	19.2%
4.	*Trichophytontansurans*	8	15.3%
5	*Mucorsp*	7	13.5%
	Total	52	100%

Table 5: Fungal Isolate and frequency of occurrence from each spoiled smoked catfish samples

S/NO	Fungal isolate	Sample A	Sample B	Sample C	Sample D	Sample E	Total
1	Mucorspp	0	2	1	3	1	7
2.	Aspergillusglaucus	1	3	4	2	1	11
3	Rhizomucorspp	1	2	3	2	2	10
4	Trichophytontonsurans	0	2	1	2	3	8
5	Aspergillusniger	1	3	3	4	5	16

Discussions

Findings of the study indicated that various fungi organism are associated to spoilage smoked catfish samples and these fungi are responsible for the degradation and micro toxin contamination of most food such as fish. The result from this study reveals the highest mean fungal count of 91.0±5.66 cfu/ml from smoked catfish obtained from Old market, however the highest value of fungal count obtained by this study is lower than those obtained by many researchers in Nigeria Adegunwa*et al*., (2018), but the result is contrary to the findings of Wage and Lasi (2019). The least fungal count was found from the smoked catfish obtained from Big gate, The range of fungal count observed in the smoked catfish samples is also in Consistence with the range of value reported by Udochukwu*et al* (2019) from smoked and fresh fish sold in Benin city, Nigeria. The result of the macroscopic and microscopic identification of the five (5) fungi species isolated from smoked catfish vended in New market, old market, small market, big gate and small gate shows the characteristics of the fungal isolates. The occurrence of *Mucorspp,Aspergilusglaucus, Rhzomucorspp*, *Trichophytontansurans* and *Aspergillusniger* in the smoked catfish was attributed by Christiana *et al*., (2020) to the fact that during storage, the fish sample reabsorbed the growth of the microorganisms in addition to the contamination during processing handling and display in the market stalls. Similarly, fungal contamination occurs probably during hawking where the fish is exposed to fungal and fungal spores contamination as reported by Dike *et al*., (2019), This as

Ekiund*et al*., (2018) puts it, that any handling of fish and the associated sanitary practice from the point of harvesting can potentially contribute to the micro flora on the final product. The result from this study revealed a highest fungal count of 91.0 ± 5.66 CFU/rnl from spoiled catfish obtained from(small gate), The least fungal count was found from the smoked catfish Obtained from (Biggate). More so, the result for the prevalence of fungalflora on the smoked catfish vended in Big gate, New market, Small market, Old market, and Small gate in Bida revealed the presence of five (5) different fungal species associated with the smoked catfish vended in the markets. *Aspergillusniger* was the most predominant with 30.7%, followed *Aspergillusglaucus* 21.2%, *Rhizomucorsp* 19.2%, *Trichophytontansurans* 15.3% and *Mucorsp* were the least abundant fungal species on the smoked catfish accounted for 13.5% each. The microorganisms isolated in this study have been reported in some fish species by Gram and Huss (2021), Dike *et al*., (2019), Akinwumi*et al*, (2018) and Udochukwu*et al*, (2019) who independently reported these organisms as the major causes of microbial spoilage of fish and the microbial count on the different media suggests contamination. This result has proven that the smoked catfish are contaminated right from the factory point. This implies that smoking is not an effective means of preservation and prevention of microbial proliferation in fish for long period of time. Furthermore, the fungal species identified in this study were aflotoxigenic fungal species that produce mycotoxins which have pathogenic effect on man. It destroys the liver and kidney resulting to death. The presence of the organisms could be a result of handling during smoking and also cross contamination during storage, after smoking and handling during sales of smoked fish as reported by Adelaja*et al*.,(2019) more so, the isolation of these fungal species as mycocontaminants of smoked fish indicated a potential health hazard as stressed by Gupte (2018).

CONCLUSION AND RECOMMENDATION

Conclusion

Evidence from the study have shown that there are fungi associated with smoked batfish and those fungi possess myctoxins that pose danger to human and animal health as they are toxic to vertebrates and other animals in low concentrations. The implication of this is that mostconsumers might have been consuming these metabolites and their prolonged intake may constitute health hazard.

Recommendation

Based on the findings of the study, it is recommended that proper care should be taken by fish vendor during processing and sell. Fish meant for consumption should be properly washed and boiled in other to kill any presence of fungi and it micro toxin.

REFERENCES

Abolagba, O., J., Adekunle, A.T., Dede, A.P. and Omoigui, G.O. (2021).Microbial Assessment of Smoked Fish (*ClariasSpp*) In Benin Metropolis, Edo State, Nigeria.Nigerian Journal ofAgriculture, Food and Environment, 7(3), 55-58.

Adegunwa A.A., Wogu M.D. and Iyavi A.D. (2018).Microflora of some smoked fish varieties in Benin City, Nigeria Ethopian Journal of Environmental Studies and Management. 4(1): 36-38.

Adelaja, O.A., Olaoye, O.J., Ikenweiwe, N.B. and Ashley-Dejo, S.S. (2019). Comparison of Microbial Load Associated with Smoked Fish (Chrysichthysnigrodigitatus) from Oyan Lake and Ogun Waterside in Ogun State, Nigeria. Global Journal of Science Frontier Research Agriculture and Veterinary. 13(8): 1-5.

Akande, G. and Tobor, J. (2019) Improved Utilization and Increased Availability of Fishing Products as an Effective Control of Aggravated Animal Protein Deficiency Induced Malnutrition in Nigeria. Proceedings of the 10th Annual Conference of the Fisheries Society of Nigeria, 18-31.

Akinwumi, F.O., Kehinde, T., Adegbehingbe, N. (2018). Microbiological Analysis of Three of Smoked Fish Obtained from the Ondo State, Nigeria. Food and Public Health. 5(4):122-126.

Akise, O.G., Abolagba, O. J. and Eyong, M. M. (2023).Mycoflora of three fish species smoke-dried using rubber wood (Heveabrassillensis) in Nigeria.Greener Journal of Agricultural Sciences. 3 (5):396-402

Christianah, I., Ayolabi, O., and Fagade, O.E. (2020).Mycological Evaluation of Smoked Fish from the Retail Outlets in Ago-JwoyeOgun State. Nigeria, Journal of Life and Physical Science. 3(2): 65-66.

Claus, D. (2018).A standardized gam-staining procedure World Journal Microbial Biotechnology.8:45 1-452.

Dike, N., Egbuobi R.C., Onyeneke, E.N., Uduji, H.I., Nwagbara, I.A., Okorie, H.M., Egbuobi, L.N. and Opara, A.U. (2019). Microbial status of smoked fish, *Scombiascombias*old in Owerri Imo state, Nigeria. African Journal of Clinical and Experimental Microbiology, 2014; 15(1): 35-39.

Eklund, M., Pebroy, G., Poysky, F., Peterson, M. and Lashbrook, L. (2018). Guidelines for Reduction and Control of *Lisrerianionocyiogens* on smoked fish. International Report on North West Fisheries Centre.

Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley, R. (2019).Description of medical fufungi. Second edition Printed in Adelaide by Nexus print Solutions, Underdale, South Australia.

Eyo, A.A. (2020). Textbook on Fish Processing Technology in the Tropics.published by National Institute for Freshwater Fisheries Research, New Bussa. University of Ilorin Press, Nigeria. Pp 403.

Fayola, M.O. and Oslo, B.A. (2019).Laboratory Manual of Microbiology.Spectrum Books Ltd; Nigeria. 34-35

Gram, L., Oundo, J.O. and Bon, J. (2019). Shelf-life of fish depends on storage temperature and initial bacterial load. Tropical Science.25: 28- 30.

Holden, M., Milr, B. and Reed, W. (2020). West Africa, Freshwater Fish. West African Nature Handbook, Longman Singapore Publishers Ltd. Singapore, 2015; 1-80.

James, G.C. and Natalie, S. (2019).Microbiology, A Laboratory Manual (10th Edition) Benjamin Cummings New York. 560.

Lasi, S., Dias, G.J. and Summers J.D. (2019). Tricothecenes In: Poultry Metabolic Disorders. Guelph Ontario, Canada, pp. 190226.

Martins, A. M. (2020). Fisheries Processing: Biochemical applications. Published by Chapman and Hall, London, 1-88.

Olokor, J.Q., Ihua, J.A., Omojono, F.S., Fabiyi, B.A and Adelomo E.Q. (2019). Hand book of practical Fisheries Technology Division National Institute of Fresh water Fisheries Research (NIFER), New Bussa, Niger State. Rem-Thomas press Pp. 13-42.

OParaku, N.F. and Mgbenka, B. O. (2022).Effect of Electric Oven and solar dryer on approximate and water activity of *Clariasgariepinus* fish.European Journal of Scientific & Research. 81:139-144.

Pepper, L.L. and Gerber, C.P. (2020). Environmental microbiology, A laboratory manual Second edition. Elsevier Academic Press Pratiwi.

Sani, M.F., Idris, A.N. and Gloria, T. (2020). Mycological Evaluation of smoked dried Fish sold at Maiduguri Metropolis Nigei.ja: Preliminary Findings and Potential Health Implications. Journal of Applied Microbiology Open Access 2 (1): 2-4.

Tobor, J. G. (2018). The Fishing Industry in Nigeria Status and Potential for Self Sufficient in Fish Production. Nigeria Institute of Oceanography And Marine Research.

Udochukwu, U., Inetianbot J., Akaba S.O. and Omorotionwan, F.O. (2019).ComparativeAssessment of the Microbiological Quality of Smoked and Fresh Fish

So1d in Benin City and Its Public Health Impact on Consumers.American Journal of Microbiological Research. 4(1): 37-40.

APPENDIX

Macroscopic examination is summarized in Table land it displays that there are various fungi organism that are found associated with spoilage of smoked catfish, it revealed colonial appearance of fungi grown from sample collected from Big gate as floccose colony resembling cotton candy, white later turns gray or grayish brown, reverse white on Sabourgard Dextrose Agar at 25°C, those from New market, as dull green with reverse white to yellow, those cultured from small market are very fluffy groh with texture of cotton candy, gray becoming dark brownish with age, reverse is white on SabourgardDextrose Agar at 25°C, those cultured from Old market are highly variable, grayish yellow, rose, or brownish surface colonies on Sabourgard Dextrose Agar at 25°C, while colonies appearance from Sample collected from small gate has black surface with white border, white cream erect hyphae colonies on Sabourgard Dextrose Agar at 25°C.

Table 2 reviews the microscopic examination and it shows that there are various morphological characteristics and identification of different species of fungi such as *Mucorspp, Aspergillusglaucus,Rhizomucorspp, Trichophvtontansurans* and *Aspergillusniger*.This result may be due to the fact that the smoked dried fish samples were processed underunhygienic condition, or the sample were contaminated as a result of their exposure in themarket place the contamination of the sample may also be attributed to the nature of package and place of storage.