ISSN:
Print - 2277 - 2755
Online - 2315 - 7453

Journal of Agricultural Science and Environment

MYCOLOGICAL EVALUATION OF DIFFERENTLY PRESERVED TILAPIA FISH IN ABEOKUTA NORTH LOCAL GOVERNMENT, NIGERIA

***JIMOH W.A[1]., IYANDA S.A[2]., ADEDEJI F.A[2]., AYELOJA A.A[1]**

[1]Department of Fisheries Technology, Federal College of Animal Health and Production Technology, Moor Plantation, PMB 5029, Ibadan. Nigeria
[2]Department of Biological Sciences, Crescent University, PMB 2104, Abeokuta, Ogun State, Nigeria
Corresponding Author: jawabus@gmail.com

ABSTRACT

Fungi load and diversity of differently preserved tilapia fish obtained from Olomoore market, Abeokuta North local Government, Nigeria were evaluated. Fish samples were purchased, differently processed (smoking, salting, freezing) and analyzed for the presence of fungi. Microbial loads on the gills and the skin of fish samples were examined and characterised using standard microbiological procedures. The progression of growth was also monitored within 10-day storage period. The fungi isolated from the differently processed tilapia were *Aspergillus niger, Aspergillus spp, Branchysporum nigrum, Candida albican, Candida spp., Fusarium solani, Fusarium spp., Paecilomyces spp., Rhizopus stolonifer* and *Aspergillus 8flavus.* No significant variation ($p>0.05$) was recorded in the fungal count of the skin during the first day of processing. However, significant variation ($p<0.05$) existed in the fungal count of the gill of the fish during the first day of processing. On the tenth day frozen fish skin had the highest fungal count while smoked fish skin possessed the lowest fungal count. There were significant difference ($p<0.05$) in the fungal count of the skin and the gill of differently processed fish samples during the storage. Similarly, significant variation ($p<0.05$) existed in the fungi count of the gill during the tenth day of processing.

Keywords: fungi load, tilapia, mycological evaluation, fungi diversity

INTRODUCTION

Tilapia fish are widely distributed and now cultured in most part of the world (Adeparusi *et al.,* 2007). Nigeria is the second largest producer of farm-raised tilapia in Africa, after Egypt (Adesulu, 1997; Fagbenro, 2002; El-Sayed, 2006; Fagbenro *et al.*, 2010). As human food, fish protein contains most of the essential amino acids in particularly lysine, methionine and tryptophan. Due to fish low cholesterol level coupled with high quality nutrient profile, it is most desirable (WFC, 2003).

However, fish, being a perishable product and a good substrate suitable for microbial growth, are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing (Beuchat 1995; De Rover 1999, Venugopal 2002). They, therefore, harbour a diverse range of micro-

organisms including plant and human pathogens from their aquatic environment (Adeparusi *et al.,* 2007, Jimoh *et al.,* 2009)**.**

Other plausible explanation to the origin of these microorganisms may be through contaminated surfaces of the processing equipment (Reij *et al*., 2004). Transfer of microorganisms by personnel during handling and preparation can also be one of the ways by which fish and its products get contaminated (Chen *et al*., 2001, Montville *et al*., 2001, Bloomfield, 2003). When fish is alive, muscle tissue is considered to be sterile, but after death, the barriers to microbiological invasion begin to break down. Fungi grow on decaying organic matters. Once they have successfully invaded fish tissues they continue to grow there and cause fish spoilage. When compared to flesh, the skin parts were found to be more vulnerable to microbial flora infection and this is because the skin is usually in direct contact with the environment (Awoniyi *et al.,* 2007). Aquatic fungi are secondary tissue invaders, which follow traumatic injuries, infectious agents or environmental insults such as poor water quality (Agbede *et al.,* 1997). *Mucor mucedo and Aspergillus niger* are known to be responsible for fish spoilage in Nigeria (Ogbondeminu and Adeniji, 1987). The predominantly common fungi species in pond fish infection in Nigeria are *Aspergillus* and *Mucor* (Okaeme, 2006). Awoniyi *et al.,* (2007) identified *Candida, Fusarium* and *Rhizopus* as well as *Saccharomyces and Variscosporum* in their study. Abolagba and Uwagbai (2011) isolated *Aspergillus niger, Mucor spp, Saccharomyces sppp, Rhizopus spp, Penicillium italicum, Neurospora spp, Cercospora spp, Candida spp,* and *Trichoderma spp.* from smoked dried *Ethmalosa fimbriata* and *Pseudotolithus elongates* sold in some markets in Edo and Delta states.

The primary objective of food processing industries is to provide safe, wholesome and acceptable food to the consumer and control of microorganisms is essential to meet this objective (Baggen-Ravn *et al*., 2003). Fish are processed in many different ways in different parts of the world. Heavy salting freezing, drying, hot smoking, canning and pasteurisation are all recognised methods of fish preservation. All affect the microorganisms on the fish in different ways and will result in a different type of microflora and different risks from spoilage organisms and pathogens. Similarly, the fish microflora load and diversity change during storage (Lund *et al*., 2000). This study therefore examines the fungi load and diversity on differently processed tilapia stored for a period of ten days.

MATERIALS AND METHODS

Collection of the Fish Samples

The fish samples (*Oreochromis niloticus)* were obtained from fishermen at Olomoore market, Abeokuta, Nigeria. They were caught from Ogun river which covers the upper Ogun to lower Ogun, flowing from Oyan dam area to Adigbe-Saraki area. They were transported to the market in baskets, and plastics containers**.** The fish were transported to the laboratory where they were properly washed and weighed. They were divided into three parts and the following treatments were given to each of them as follows as shown in table 1.

on appropriate agar slants as stock culture. Microscopic examination of young, actively growing moulds was on the basis of structures bearing spores and on the spore themselves; presence or absence of septation, rhizoid or other tissues. The fungi isolates were identified by their micro-morphology as well as the colour and micro-morphology of their sporulating structures and conidia according

to Onions *et al.,* (1981)

Fungal Count: Colonies which developed after incubation were subjected to counting. The total fungal counts were expressed as spore/g.

Statistical Analysis

Results are expressed as mean ± SD. All data were subjected to one way ANOVA using SPSS 13.0 for window software. Where significant differences occurred, the group means were further compared with Duncan's multiple range test using SPSS 13.0 (SPSS, IL, USA).

RESULTS

Table 2 shows the fungi isolates from the skin and gills of differently processed *Oreochromis niloticus* obtained from Olomoore market, Abeokuta. The fungi isolated were *Aspergillus niger, Aspergillus spp, Branchysporum nigrum, Candida albican, Candida spp., Fusarium solani, Fusarium Spp., Paecilomyces spp., Rhizopus stolonifer, Aspergillus flavus.*

Table 3 shows the fungi count of differently processed tilapia. There is no significant difference ($p>0.05$) in the fungi count of the skin during the first day of processing. However, significant variation ($p<0.05$) existed in the fungi count of the gill of the fish during the first day of processing. In the second day, smoked fish gill had the highest fungi count while frozen fish gill had the lowest fungi count there is significant difference in the fungi count of differently processed gill during the second day of processing. However, significant variation ($p<0.05$) existed in the fungi count of the skin of the fish during the second day of processing. In the fourth day, salted fish gill had the highest fungi count while frozen fish gill had the lowest fungi count. However, there is significant variation ($p<0.05$) in the fungi count of the skin of the fish during the fourth day of processing. In the eighth day frozen fish skin had the highest fungi count while salted fish skin had the lowest fungi count there is significant difference in the fungi count of differently processed fish during the eighth day of processing. However, significant variation ($p<0.05$) existed in the fungi count of the gill during the eighth day of processing. In the tenth day frozen fish skin had the highest fungi count while smoked fish skin had the lowest fungi count. There is significant difference ($P<0.05$) in the fungi count of differently processed fish during the tenth day of processing. So also, significant variation ($p<0.05$) existed in the fungi count of the gill during the tenth day of storage.

Table 2: IDENTIFICATION OF FUNGI ISOLATES FROM THE SKIN AND GILLS OF DIFFERENTLY PROCESSED *Oreochromis niloticus* OBTAINED FROM OLOMOORE MARKET, ABEOKUTA

Days	Processing		Skin	Gill
Day 0	Fresh Fish	Cultural characteristics	Black background colour with white spores	Black background colour with white spores
		Spore conidia under microscope	Conidia heads radiate. Conidiophores stripes smooth-walled with one cell and vesicle, non septate, non columella	Conidia heads radiate. Conidiophores stripes smooth-walled with one cell and vesicle, non septate, non columella
		Probable Fungi Isolates	Aspergillus niger	Aspergillus niger
Day 2	Smoked Fish	Cultural characteristics	White background with black and white spores	White background with black and white spores
		Spore conidia under microscope	Non-septate hyphae and coenocytic twin sporangiophore. Spores are of various shape but musty oval	Non-septate hyphae and coenocytic twin sporangiophore. Spores are of various shape but musty oval
		Probable Fungi Isolates	Rhizopus stolonifer	Rhizopus stolonifer
	Salted Fish	Cultural characteristics	Black background with white and black spores.	Colonies with black background and white spores
		Spore conidia under microscope	Conidiophores upright, simple terminating in a globose. Conidia is one cell and globose	Conidiophores upright, simple terminating in a globose. Conidia is one cell and globose
		Probable Fungi Isolates	Fusarium spp	Fusarium spp
	Frozen fish	Cultural characteristics	Green background with white spores	Black background with white spores
		Spore conidia under microscope	Conidiophores upright, Spores are of various shape	Conidia heads radiate. Conidiophores stripes smooth-walled with one cell and vesicle, non septate, non columella
		Probable Fungi Isolates	Candida spp	Aspergillus niger
Day 4	Smoked Fish	Cultural characteristics	Black background with black spores	Green background with green spores
		Spore conidia under microscope	Conidiophores pale at the apex and cluster septate. Conidia unequally with 3-4 cell	Conidiophores upright, Spores are of various shape
		Probable Fungi Isolates	Branchysporum nigrum	Candida spp
	Salted Fish	Cultural characteristics	Black background with black spores and Green background with green spores	Green background with green spores
		Spore conidia under microscope	Conidiophores pale at the apex and cluster septate. Conidia unequally with 3-4 cell; Conidiophores upright, Spores are of various shape	Conidiophores upright, Spores are of various shape

Continuation of Table 2

		Probable Fungi Isolates	Branchysporum nigrum;Aspergilus flavus	Branchysporum nigrum
Day 6	Smoked Fish	Cultural characteristics	Black background with green and white spores	Green background with green, white and black spores
		Spore conidia under microscope	Conidia heads radiate. Conidiophores stripes smooth-walled with one cell and vesicle, non septate, non columella	Mycelium are not extensive, one cell forming short chain by budding
		Probable Fungi Isolates	Aspergillus spp	Candida albican
	Salted Fish	Cultural characteristics	Black background with green and white spores	Green background with green and white spores
		Spore conidia under microscope	Conidia heads radiate. Conidiophores stripes smooth-walled with one cell and vesicle, non septate, non columella	Mycelium are not extensive, one cell forming short chain by budding
		Probable Fungi Isolates	Aspergillus niger	Candida albican
	Frozen fish	Cultural characteristics	Green background with green spores	Green background with green spores
		Spore conidia under microscope	Mycelium are not extensive, one cell forming short chain by budding	Mycelium are not extensive, one cell forming short chain by budding
		Probable Fungi Isolates	Candida albican	Candida albican
Day 8	Smoked Fish	Cultural characteristics	Black background with green and white spores	Black background with green and white spores
		Spore conidia under microscope	Conidia heads radiate. Conidiophores stripes smooth-walled with one cell and vesicle, non septate, non columella	Conidia heads radiate. Conidiophores stripes smooth-walled with one cell and vesicle, non septate, non columella
		Probable Fungi Isolates	Aspergillus niger	Aspergillus niger
	Salted Fish	Cultural characteristics	Green background with green and white spores	Green background with green spores
		Spore conidia under microscope	Mycelium are not extensive, one cell forming short chain by budding	Mycelium are not extensive, one cell forming short chain by budding
		Probable Fungi Isolates	Candida albican	Candida albican
	Frozen fish	Cultural characteristics	Black background with white and black spores.	Black background with black spores, white background with white spores,

Continuation of Table 2

		Spore conidia under microscope	Conidiophores pale at the apex and cluster septate. Conidia unequally with 3-4 cell	Conidiophores pale at the apex and cluster septate. Conidia unequally with 3-4 cell. Conidiophores upright, simple terminating in a globose. Conidia is one cell and globose
		Probable Fungi Isolates	Branchysporum nigrum	Branchysporum nigrum, Fusarium solani
Day 10	Smoked Fish	Cultural characteristics	Brown background with white and black spores	Green and black spores with no background colour, green background with green and black spores.
		Spore conidia under microscope	Conidiophores are more divergence, one cell ovoid to fusoid	Mycelium are not extensive, one cell forming short chain by budding
		Probable Fungi Isolates	Paecilomyces spp	Candida spp
	Salted Fish	Cultural characteristics	Green background with brown, green and black colour.	Black spore with no background colour, green background with green and black spores
		Spore conidia under microscope	Coniophore upright simple terminate in a globose, bearing phialides at the apex. Conidia is one cell, globose	Conidiophores pale at the apex and cluster septate. Conidia unequally with 3-4 cell
		Probable Fungi Isolates	Aspergillus spp	Branchysporum nigrum
	Frozen fish	Cultural characteristics	Green background with green spores	Green background with green and black spores
		Spore conidia under microscope	Mycelium are not extensive, one cell forming short chain by budding	Mycelium are not extensive, one cell forming short chain by budding
		Probable Fungi Isolates	Candida albican	Candida spp

Table 3: The Fungi Count (spore/g) of the Differently Processed Tilapia Obtained from Olomoore Market, Abeokuta

	Day 0		Day 2		Day 4		Day 6		Day 8		Day 10	
	Gill	Skin	Gill	Skin	Gill	Skin	Gill	Skin	Gill	Skin	Gill	Skin
Smoked x 10-6	1.0 ± 0.01c	1.5 ± 0.01	5.95±0.01 a	4.9 ± 0.01b	3.95±0.01 b	3.1 ± 0.01c	4.1 ± 0.01c	2.9± 0.01b	5.1 ± 0.01c	1.8 ± 0.01b	1.8 ± 0.01c	4.95 ± 0.01c
Salted x 10-6	2.5 ± 0.01a	2.4 ± 0.01	2.5± 0.01b	3.9 ± 0.01c	7.9 ± 0.01a	4.9 ± 0.01a	4.9± 0.01b	7.95±0.01 a	5.95±0.01 b	1.1 ± 0.01c	1.1 ± 0.01c	5.95 ± 0.01b
Frozen x 10-6	2. ± 0.01b	2.1 ± 0.01	1.1 ± 0.01c	7.8 ± 0.01a	2.1 ± 0.01c	3.9± 0.01b	3.95±0.01c	2.1 ± 0.01c	7.1 ± 0.01a	9.1 ± 0.01a	8.5 ± 0.01b	6.95 ± 0.01a

Means with the same superscript along the same column are not significantly different (p>0.05)

DISCUSSION AND CONCLUSION

Fungal tests on fish and fish products are used by the industry for contractual and internal purposes and by the authorities to check that the microbiological status is satisfactory (Jay 1992). In this present study, The isolated fungi spp. were *Aspergillus fumigates, Fusarium solani, Brachysporum nijrum, Aspergilus niger, Candida albican, Penicilium italicum, Aspergillus flavus, Paecilomyces spp, Rhizopus stolonifer.* Most of the fungi found on these processed fish are those commonly found in soil and water. The fungi isolated in this present study is similar to the microorganisms reported by Olawale *et al.* (2005) and Adesokan *et al*. (2005). *Aspergillus niger* obtained in the fish samples were in accordance with Martin (1994) when he stated that these organisms were the commonest fungi associated with processed fish and these microorganisms were also reported by Abolagba and Igbinevbo (2010) in smoked fish (*Clarias sp*) sold in Benin metropolis. The results could not establish whether contamination took place before the raw material was being processed. Plausible explanation that could be given is that contamination did take place in the processing area, as this is supported by other studies. According to Venugopal (2002) contamination of fish particularly by pathogens may occur prior to harvest, during capture, processing, distribution and/or storage. Other studies dealing with different processing operations have similarly concluded that the plant and processing environment is the source of product contamination rather than the raw material. However, this does not exclude the possibility that the raw fish or material is an important initial source for contaminating processing equipment and environment (Vogel *et al*. 2001). Also, water, like food, is a vehicle for the transmission of many agents of diseases (Kirby *et al*. 2003, Jimoh *et al,* 2009). The occurrence of *Aspergillus sp, Rhizopus sp, and Penicillium sp* could be due to the fact that during storage, the fish sample reabsorbed moisture from the environment which then supported the growth of the microorganisms, in addition to the contamination during processing, handling and display on the market stalls. (Christianah *et al.,* 2010). It is therefore suggested that consumers should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of microbial contamination. However, the processors/handlers/sellers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contamination of these processed frozen seafood products. In addition caution should be taken in consuming processed fish shaded openly because such fish could contain microbial cells and reheating may be necessary to destroy or inactivate such cells.

REFERENCES

Abolagba, O.J., O.O. Melle, 2008. Chemical composition and keeping qualities of a Scaly Fish Tilapia (Oreochromis niloticus) Smoked with two Energy Sources. African Journal of General Agriculture, KLOBEX, 4 (2): 113-117.

Abolagba, O.J., Igbinevbo, E.E. 2010. Microbial Load of Smoked Fish (*Clarias sp)* Marketed in Benin Metropolis, Nigeria. *Research Journal of Fisheries and Hydrobiology*, 5(2): 99-104.

Abolagba, O.J., Uwagbai, E.C 2011. A comparative analysis of the microbial load of smoke-dried fishes (*Ethmalosa fimbriata* and *Pseudotolithus elongatus*) sold in Oba and Koko markets in Edo and Delta states, Nigeria at different seasons 5(5): 544-550

Adeparusi, E. O., Afolabi, G. O., Jeff-Agboola, Y, A. 2007. Bacterial and Fungi Flora of Wild *Oreochromis niloticus* and *Clarias gariepinus* collected from Owena Reservoir, Western Nigeria. *Advances in Food,* 29 (2):162-166

Adesokan I. A., Ogunbanwo S. T., Odetoyinbo, B. B. 2005. Microbiological quality of selected brands of beer in Nigeria. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, from 6-10th November. p. 21.

Adesulu, E. A. 1997. Current status of tilapia in Nigerian aquaculture. *Proceedings of the Fourth International Symposium on Tilapia Aquaculture,* (K. Fitzsimmons, ed.). Orlando, USA. pp. 577-583

Agbede, S. A., Olufemi, B. E., Adeyemi, A. O. 1997. Preventive veterinary medicine: shell fish hygiene. *Proceeding and abstract, 34th Annual National Congress of Nigerian Medical Association,* 32-36.

Awoniyi, T. A. M., Bello-Olusoji, O. A ., Adeparusi, E.O. 2007. Microbial occurrence in pond-raised *Clarias gariepinus* and *Oreochromis niloticus* in tropical humid South West Nigeria. *Advances in Food Science*, 29(2): 90-93

Bagge-Ravn, D., Ng, Y., Hjelm, M., Christiansen, N.J., Johansen, C., Gram, L. 2003. The microbial ecology of processing equipment in different fish industries -analysis of the micro flora during processing and following cleaning and disinfection. *International Journal of Food Microbiology*. 87: 239 -250.

Bloomfield, S.F. 2003. Home Hygiene: a risk approach. *International Journal of Hygiene and Environmental Health*. 206: 1-8.

Beuchat, L. R. 1995**.** Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection*. 59: 204-216.

Chen, Y. H., Jackson, K. M., Chea, F. P., Schafftner, D. W. 2001. Quantification and variability analysis of bacterial cross contamination rates in common food service tasks. *Journal of Food Protection*. 64: 72-80.

De-Roever, C. 1999. Microbiological safety evaluations and recommendations on fresh produce. *Food Control.* 9: 321-347.

Christianah, I. Ayolabi., Fagade, O. E. 2010. Mycological Evaluation of Smoked Fish from the Retail Outlets in Ago-Iwoye, Ogun State, Nigeria. *Journal of Life and Physical Science.* 3(2): 65-66.

El-Sayed, A. F. 2006. *Tilapia Culture* CABI Publications, Wallingford, UK. 257pp.

Fagbenro, O. A. 2002. Tilapia: fish for thought. 32nd Inaugural Lecture, Federal University of Technology, Akure, Nigeria. 77pp.

Fagbenro, O. A., Jegede, T., Fasasi. O. S. 2010. Tilapia aquaculture in Nigeria. *Applied Tropical Agriculture* 15: 49-55.

Jay, J. M. 1992. Modern Food Microbiology. Microbiological Indicators of food safety and quality, Principles and Quality control, and microbiological criteria. New York: Van Nostrand Reinhold.

Jimoh W. A, Jabar M. B ., Adeleke M. A., Bello B. K. 2009. Diversity and microbial load of fungi isolates of the gut sections of captured and cultured *Clarias gariepinus* in Abeokuta South Western Nigeria. *Journal of Field Aquatic Studies, Nigeria,* 5: 54-60

Kirby, R. M., Bartram, B., Carr, R. 2003. Water in food production and processing-Quality and quality concerns. *Food Control*. 14: 283-299.

Lund, B. M., Baird-Parker, T. C., Gould, G. W. (Eds) 2000. The Microbiological Safety and Quality of Food. Vol. 1. Aspen Publishers Inc. Gaithersburg, Maryland.

Martins, A. M., 1994. Fisheries Processing: Biochemical Applications. Published by Chapman and Hall, London. pp: 1-88.

Montville, R., Chen, Y., Schaffner, D. W. 2001. Determination of bacterial cross-contamination rates from hand to food through a glove barrier. *Journal of Food Protection*. 64: 845-849.

Onions, A. H. S., Allsopp., Eggins H. O. W. 1981. Smiths Introduction to Industrial Mycology, Edward Arnold, London 389p

Olawale, A. K., Oluduro A. O., Famurewa O. 2005. Evaluation of microbiological and sanitary standards of canteens and eateries in Osun State Polytechnic, Iree. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), UNAAB, Abeokuta, from 6-10th November. p.19.

Ogbondeminu, E. S., Adeniji, H. A. 1987. Microbial ecology of aquaculture system. Changes in population of of bacteria communitiesin a fertilized fish pond in Lake Kainji area. ECOSON 187-195pp.

Okaeme, A. N. 2006. Fish diseases; prevention and control. Paper presented at the VCN professional continuing Education Seminar, Akure, March , 2006, 1-17.

Reij, M.W., Den Aantrekker, E.D., ILSI Europe Risk Analysis in Microbiology Task Force 2004**.** Recontamination as a source of pathogens in processed foods. *International Journal of Food Microbiology*. 91:1-11.

Venugopal, V. 2002. Biosensors in fish production and quality control. *Biosensors and Bioelectronics*. 17: 147-157.

Vogel, B. F., Huss, H. H., Ojeniyi, B., 2001. Elucidation of Listeria monocytogenes contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. *Applied and Environmental Microbiology* 67 (6): 2586-2595.

World Fish Centre (WFC) 2003. An issue for everyone. A concept paper for 'Fish for All' produced by World Fish Centre, Penang, Malaysia, 2-4.

(Manuscript received: 14th March, 2012; accepted: 3rd April, 2013).