



DETERMINATION OF MICROBIAL LOAD OF SELECTED SMOKED FISH SOLD IN ABRAKA MARKETS DELTA STATE

Anie, C. O.*¹, Jemikalajah, D. J.² and Eke, C. N.¹

¹ Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria.

² Department of Microbiology, Faculty of Science Delta State University Abraka. Nigeria

ABSTRACT

Fish is a common source of protein available to man and it is highly consumed due to its immense health benefits and its affordable price. This research investigated the microbiological quality, the presence and the most prevalent organisms in smoked fish that were obtained from six fish sellers in two major markets, big and small market in Abraka Delta State, south- south Nigeria. The samples were microbiologically analyzed for viable bacteria and fungi growth on Mannitol salt agar, MacConkey agar, Tryptic soy agar and Sabourand dextrose agar using the spread plate technique. The mean microbial viable count obtained ranged from 1.6×10^9 cfu/ml- 2.4×10^{10} cfu/ml. The different fishes analyzed were found to contain contaminating organisms which were microscopically and biochemically identified to be bacteria such as *Staphylococcus aureus*, *Staphylococcus epidemis*, *Klebsiella spp*, *Bacillus subtilis*, *Micrococcus spp*, *Aeromonas spp*, *Citrobacter spp*, *Serratia spp*, *Shigella spp* and *Micrococcus spp* and fungi such as *Aspergillus spp*, *Cladosporium spp* and *Scopulariopsis spp*. This high level of fish contamination can be due to poor hygiene practices of the fish vendors during preparation and sale of the smoked fish, poor storage processes and also the environment to which the fish is exposed to during smoking. To prevent the spread of organisms that are of public health importance, the processing, storage and sale of smoked fish should be carried out under good sanitary conditions.

Keywords: Smoked Fish, Abraka, Hygiene, Bacteria, Fungi

Introduction

Fish is known to be a perishable food item that normally deteriorates immediately after harvest at very high ambient temperature (Aberoumand, 2010) so, it has to be preserved immediately. The high ambient temperature and humidity of Nigeria climate usually speed up spoilage processes in harvested fishes. The speed of spoilage is related to the initial bacterial load. The higher the count, the sooner the spoilage occurs (Saliu 2008). Microbial content of fish is

Corresponding Author: Anie, C. O. Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, Abraka, Delta State.
Phone: +2348066390868; Email: oliseloke@yahoo.com



largely determined by the fish microbial flora. It poses danger if fish harvested from polluted aquatic habit is not properly processed before eaten or eaten raw. Numerous bacteria that have great potential to spoil proliferate in the slime surfaces, intestines and gills of live fish however their naturally possessed defenses mechanisms prevent the invasion while the fish is still alive. Proliferation and invasion usually occurs as soon as fish dies (Agbolagba and Uwagbai, 2011). Okareh and Erhahon (2015) have reported that *Staphylococcus spp* usually have pathogenic strains that are capable of causing food poisoning because of the thermo stable *Staphylococcus enterotoxin* that is resistant to gastrointestinal enzymes. The presence of *Aspergillus fumigatus* and *Aspergillus flavus* in fish raises a great health worry as of their mycotoxigenic potentials. Species of *Aspergillus flavus* and *Aspergillus fumigatus* have been discovered to produce aflatoxins, which is capable of destroying the kidney and the liver in humans leading to death. The occurrence of these organisms in the fish could result from inadequate handling methods during the period of smoking and cross contamination during period storage, or even at the time sales of smoked fish (Essien *et al.*, 2005). Fungi spp have been isolated fish samples such as stockfish and smoked fish (Junaid *et al.*, 2010). Nevertheless, a number of methods exist in

prevention of the proliferation and growth of pathogenic organisms at the time of storage and distribution of processed fish. To decrease the loss that is connected to spoilage, preservation techniques which include sun drying, salting, freezing, cold storage, fermentation, oven-drying, and smoking are employed (Asiedu and Sanni, 2002).

About 66% of preserved fish in Nigeria are smoked (Tawari and Abowei, 2011). Smoking is mostly desired partly owing to the ease of the practice, and consumer prefers the taste that wood smoke gives to the fish which is such a desirable taste. It dehydrates and makes the fish muscle to be tough which provides a longer shelf life, lowers the pH and thereby making it less susceptible to spoilage (Sengor *et al.*, 2004; Olorok *et al.*, 2007)

Microorganisms that found within smoked fish are usually found in soil, decaying organic matter and improperly smoked or contamination during handling.

The microorganisms connected with smoked fish are typically those of the normal flora of the fish and that of human body, *Mucor spp* even the fungi that are airborne and that of the soil. These organisms including *Bacillus brevis* are normal bacteria present in fish (Cheikyula and Awobode, 2014). *Staphylococcus aureus*, *Neisseria sicca*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Salmonella spp*,

Micrococcus luteus, *Bacillus subtilis*, *Escherichia coli* *Proteus spp*, *Enterobacter aerogenes* are usually the main bacteria that are isolated often associated with smoked fish. These microorganisms may have found their way into the fish through hand to hand contact. Apart from *Bacillus brevis*, the other bacteria are a subset of the normal flora found on the human skin. *Aspergillus niger*, *Spiromyces minutes*, *Geotrichum albidum*, *Aspergillus flavus*, *Aspergillus fumigatus* are some fungi isolates associated with smoked fish (Akinwumi and Adegbehingbe, 2015).

In scores of developing communities like Abraka in Ethiope East Local Government Area of Delta State, Nigeria, smoked fish are habitually hawked without considering the probability of the microbial contamination from the environment. The present study is therefore aimed at assessing the microbial load of smoked mackerel fish sold in Abraka markets.

MATERIALS AND METHOD

Glass wares: Conical flasks, McCartney bottles, Measuring cylinder, Durham tubes, Test tubes, Beakers, L-shaped stirring rod, Petri dishes, Pipettes, Microscopic slides.

Equipment: Autoclave, Incubators, Weighing balance, Centrifuge, Microscope, Refrigerator, and Bunsen burners.

Bacteriological Media:

Nutrient agar, MacConkey agar, Sabourand dextrose agar, Mannitol agar, Tryptic soy agar, Simmon citrate agar, Urea broth, Nutrient broth, Glucose, Sucrose, Lactose, Oxidase reagent, Kovac's reagent, Lactophenol blue, Phenol Red, Lugol iodine, Crystal violet, Safranin, Peptone water, Hydrogen peroxide, Sterile water, Serum.

Miscellaneous:

Spatula, syringes, wire loops, EDTA blood collection tubes, test tube racks, cotton wool, marker pen, writing pad, lead acetate paper, sterile knife, aluminum foil, masking tape, ethanol, face mask, bleach, napkin, headgear, Detergent, hand gloves,

Collection of samples

Three samples of Mackerel fish four days old each were collected from Six (6) randomly selected fish stalls in the two major markets labeled STA, STB,STC,STD,STE and STF from the big and small markets in Abraka, Delta state Nigeria.

Microbiological examinations

The table was swabbed using disinfectant (70% alcohol) and the media used were prepared and sterilized according to manufacturers' specifications on their labels. The fish samples were analyzed microbiologically using the spread plate technique but first.

Viable count

Ten gram (10 g) of fish sample was cut with a sterile knife and weighed. This was crushed in a sterile 250ml beaker using a sterile spoon and 90ml of sterile water was added. The mixture was

homogenized using the spoon under aseptic condition.

Seven test tubes were filled with 9ml sterile water and a serial dilution of the homogenized fish sample was made to give 10^{-7} dilution. 1ml was discarded from the 7th test tube and 0.1ml of the 7th dilution was used for the spread plate technique (Cheesbrough, 2006).

Spread Plate Technique

The Petri dishes were labeled according to the code name given to the fish sample by the researcher and the culture media (mannitol agar, tryptic soy agar, maConkey agar, and sabourand dextrose agar). The sterilized media were poured aseptically into each Petri dish. A 2ml syringe was used to take 0.1ml of the homogenized fish sample dilution on each of the solidified media in the Petri dishes. An L-shaped glass spreader was dipped into a solution of ethanol, flamed and was used to spread the solution on the surface of the solidified agar in the Petri dish.

The Petri dish was covered, inverted and incubated at 37°C for 24 hours and 48 hours respectively (for bacteria growth). For the fungal growth, the Petri-dish was placed in a microaerophyll condition for 24 hours, 72 hours and 120 hours respectively. For the bacteria, growth was checked for the first day and second day and the colonies formed were recorded while for the fungi, the growth was checked for the first day, third day and fifth day and the colonies formed were recorded accordingly. The isolates were then sub cultured into slants (Cheesbrough, 2006).

Sub-culturing of isolates

An overnight broth was prepared in bijou bottles from the isolates of the organisms that were aforetime prepared into slants. The Petri dish was labeled according to the labeled information of the fish sample on each slant, MacConkey agar, Sabourand dextrose agar, Tryptic Soy agar and Mannitol salt agar respectively. The agar media aforementioned were prepared according to manufacturers' direction and aseptically dispensed into each Petri dish respectively. They were allowed to solidify. The wire loop was flamed and used to take a loop full from the overnight broth bottle and used to streak the solidified agar in the Petri dish respectively.

The Petri dish was covered, inverted and incubated at 37°C for 24 hours (for bacteria growth). For fungal growth, the Petri dish was placed in a microaerophyll condition for 24 hours, 72 hours, and was matched with a fungi chart after microscopic examination. For the bacteria, growth was checked for the first day, while for the fungi, the growth was checked after 72 hours (Cheesbrough, 2006)

Identification of Microbial Isolates

After incubation, bacteria and fungi were identified based on the cultural, morphological and reaction to biochemical test (Cheesbrough, 2006).

Microscopic examination (Lactophenol Test)

The slide was wiped with alcohol soaked cotton wool to degrease it. Thereafter a sterile nichrome needle was used to collect the organism (fungus)

and teased on the slide. Lactophenol was dropped on the teased fungus and the slide was covered with a cover slip and then placed under the microscope for examination. The magnification used for viewing it was x40 (Beneke and Stevenson, 1977).

The data obtained was analyzed statistically using mean and simple percentages.

RESULTS

Our results showed mean viable microbial colony counts (cfu/ml) of 1.6×10^9 , 2.0×10^9 , 8.3×10^9 , 2.3×10^{10} , 4.7×10^{10} , 23.6×10^{10} for STE, STA, STC, STB, STD, and STF in ascending order respectively (Table 1). The highest mean microbial colony count was obtained from STF and the least in STE.

Table 1: Mean values of total bacteria colony count (cfu/ml)

Fish Stalls	Viable microbial count (cfu/ml)
STA	2.0×10^9
STB	2.3×10^{10}
STC	8.3×10^9
STD	4.7×10^{10}
STE	1.6×10^9
STF	24.0×10^{10}

Key: ST = Stall

The bacterial isolates identified in this study was *Staphylococcus aureus*, *Staphylococcus epidermis*, *Micrococcus spp*, *Citrobacta spp*, *Klebsiella spp*, *Bacillus substilis*, *Aeromonos spp* and *Serratia spp*. This shows that both Gram positive and Gram negative bacteria are contaminations in the fish (Table 2).

Table 2: Percentage occurrence of different bacterial isolates

Bacterial Isolates	N0. of Isolates	% occurrence
<i>Staphylococcus aureus</i>	5	25
<i>Staphylococcus epidermis</i>	2	10
<i>Bacillus substilis</i>	2	10
<i>Micro luteus</i>	1	5
<i>Aeromonas spp</i>	1	5
<i>Citrobacta spp</i>	2	10
<i>Serratia spp</i>	3	15
<i>Shigella spp</i>	1	5
<i>Micrococcus spp</i>	2	10
<i>Klebsiella spp</i>	1	5

The most prevalent fungi organism observed was *Aspergillus spp* (38.5%), *Scopulariopsis spp* (36.5%), while the least was *Cladiorium spp* with 25% occurrence (Table 3).

Table 3: Percentage occurrence of different fungal isolates

Fungal isolates	N0. of isolates	% occurrence
<i>Cladiorium spp</i>	2	25.0
<i>Aspergillus spp</i>	3	38.5
<i>Scopulariopsis spp</i>	3	36.5
Total	8	100

DISCUSSION

The study showed that *Staphylococcus aureus* was the most implicated organism which accounted for 25% while the least were *Aeromonos spp*,

Micrococcus spp, *Shigella spp* and *Klebsiella spp* which accounted for 5% each. The high count of bacteria growth can be due to the handling technique of the fish by the seller before and after smoking. This is in agreement with (Maga, 1988) who considered smoking a mild preservative treatment which kills and prevents microbial growth due to combined effect of heating, drying, pH and antimicrobial smoke components. Therefore, smoking does not completely eradicate microbial load of fish because the fish already has high microbial load due to its habitat which is water. Also exposure of the fish to environmental factors like sun and the atmosphere during sale contributes to more possibilities of contamination. This supports the observation of (Eklund *et al.*, 1993) who reported that handling conditions and poor sanitary practices right from harvesting through processing could contribute to the micro flora on the final product.

The presence of *Klebsiella spp* is an indication of fecal contamination and this is in line with the report of Frazier and Westhoff (1995) which states that the microbial flora of fish also depends on the microbial contents of the water where they lived.

All samples of fish analyzed showed fungal growth after they were kept in a microaerophilic condition for duration of 72 hours. They were all moulds (*Aspergillus spp*, *Cladosporium spp* and *Scopulariopsis spp*.) and they are known to cause food spoilage. The fungi isolated from these fish were similar to those identified by Junaid *et al* (2010) in their studies on smoked fish and stockfish. These fungi can also be found in soil and water with which

the fish is in contact as earlier opined by Abbey (2007). The isolation of these fungi further show that smoked fish still has reasonable amount of moisture left even after the smoking procedure. This is in agreement with Schewan (1977) who attributed species of fungi observed in smoked fish sample to microbial spoilage as a result of partial drying and incomplete removal of moisture during smoking.

CONCLUSION

The present study has shown that smoked fish obtained from Abraka markets were contaminated. This implies that fish smoking is not an effective method for preservation and prevention of microbial multiplication in fish. It is therefore imperative for fish processors and vendors to maintain high standard of hygiene and food handling techniques in order to reduce fish contamination in the studied area.

CONFLICT OF INTEREST: None

REFERENCES

- Aberoumand, A. (2010). Estimation of microbiological variations in minced lean fish products. *World Journal Fish and Marine Science*. 2: 204 – 207.
- Saliu J.K. (2008). Effect of smoking and frozen storage on the nutrient composition of some African fish. *Adv. Nat. App. Sci*. 2(1): 16-20.
- Asiedu, M. and Sanni, A.I. (2002). Chemical composition and microbiological changes during spontaneous and starter culture fermentation of Enam Ne-Setaakye, a West African fermented fish-carbohydrate product. *European Food Research Technology*, 215: 8-12.
- Agbolagba, O.J. and Uwagbai, E.C. (2011). A comparative analysis of the microbial load of smoked dried fishes (*Ethmalosa fimbriata* and *Pseudotolithus elongatus*) sold in Oba and

- Koko markets in Edo and Delta states Nigeria at different seasons. *Australian Journal Basic and Applied Sciences*, 5(5), 544-550.
- Abolagba, O. J., and Melle, O. O. (2008). Chemical composition and keeping qualities of a Scaly Fish Tilapia (*Oreochromis niloticus*) Smoked with two Energy Sources. *African Journal General Agriculture*, 4: 113-117.
- Cobiac, L., Clifton, P. M., Abbey, M., Belling and Nestel, O. (1991). Fish oil 1– 3 fatty acid in mildly hyperlipidemic males. *American Journal of Clinical Nutrition*. 53:1210- 1216
- Tawari C.C. and Abowei, J.F.N. (2011). Traditional fish handling and preservation in Nigeria. *Asian Journal Agricultural Science* 3: 427-436
- Agbolagba, O.J. and Iyeru, O.A. (1998). Study of insect pest infecting traditionally processed fish sold in Benin City metropolis. *Nigerian Journal of Applied Science*, 16:25-29
- Olorokun J.O, Ihuahi J.A, Omojono F.S, Fabiyi B.A and Adelomo, E.O. (2007). Hand book of practical Fisheries Technology Division. National Institute of Fresh water Fisheries Research (NIFFR), New Bussa, Niger State. Rem-Thomas press pp 13-42.
- Junaid, S.A., Olauboin, F. and Olabode, A.O. (2010): Mycotic contamination of stock fish sold in Jos, Nigeria. *Journal of Yeast and Fungal Research*, 1(7): 136
- Hohl, TM, and Feldmesser, M. (2007). *Aspergillus fumigatus*: principles of pathogenesis and host defense. 6 :1953-63.
- Tyokumbur, J. Cheikyula, H. And Awobode, O. (2014). Microbial Flora and Nutrient Content of Market Bought Smoked African Cat Fish *Clarias gariepinus* from Jos, Nigeria. *Food Science and Quality Management*.32,
- Akinwumi, F. O, and Adegbehingbe, K. T. (2015) Microbiological Analysis of Three of Smoked Fish Obtained from the Ondo State, Nigeria *Food and Public Health*. 5: 122-126
- Beneke, E.S. and Stevenson, K.E (1977). Classification of food and beverage fungi. In: Beuchat, L.R (ed): Food and beverage mycology. AVI publishing company. Inc. Westport, Connecticut. P1
- Shewan, J. M. (2000). The microbiology of sea water fish. In: Fish as Food. (Ed. Borgstrom, I.) New York Academic press. Inc; 2000, 487.
- Teugels, G. G. and Audenerde, D. F. E. (2003). Microbiological Analysis of Three of Smoked Fish Obtained from the Ondo State, Nigeria. *Food and Public Health*. 2: 521- 600
- Adelaja, O.A., Olaoye O. J., Ikenweibe N. B., and Ashley-Dejo, S. S. (2013). Comparison of microbial load associated with smoked fish (*Chrysichthys nigrodigitatus*) from Oyan Lake and Ogun Waterside in Ogun State, Nigeria. *Global Journal Science Frontier Research Agriculture and Veterinary*, 13: 34-39.
- Adedeji, O. B. and Ibrahim S. O., (2011). Assessment of Microbial safety of fresh shrimps offered for sale at Alesinloye and Eleyele markets in Ibadan, southwestern. *Nigeria Journal of Applied Science in Environmental Sanitation*. 6: 239-346
- Okareh O.T. and Erhahon O.O. (2015). Microbiological Assessment of Food and Hand-Swabs Samples of School Food Vendors in Benin City, Nigeria, *Food and Public Health*. 5: 23-28
- Herman, H. W., Lin, P. J. Petrecca, M. R., Simmons, and Houghton J. (2011). Centrifugal bioreactors and their application in remediations. *Remediation Journal*. 11(4): 15-33.
- Alexander, B. and Austin B. (1986). Bacterial microflora associated with a commercial fish smoker. *FEMS Microbiology Letter*. 34: 309-312.
- Maga J.A. (1988). Smoke in Food processing. *Journal of Food Science* 6:26-35
- Essien, J. P., M. A., Ekpo, and Brooks, A. A., (2005). Mycotoxigenic and proteolytic potential of moulds associated with smoked shark fish (*Chlamydoselachus anguincus*). *Journal of Applied Science. Environmental Management*. 9: 53-57.
- Cheesbrough, M. (2006). District laboratory practice in tropical countries Part 2, (2nd Edition) Cambridge University Press.
- Agbolagba O.J., and Igbinvbo, V.E. (2010). Microbial load of fresh and smoked fish

marketed in Benin metropolis, *Nigerian Research Journal of Fisheries and Hyrobiology*, 5: 99-104.

Eklund, M., Pebroy, G., Poysky, F., Peterson, M. and Lashbrook, L. (1993). Guidelines for reduction and control of *Listeria Monocytogens* on smoked fish. International Reports On North West Fisheries Center.

Frazier, W.C. and Weasthoff, D.C. (1995). *Food Microbiology*, (8thed.) MC: New York, 1995.57-62

Abey, S.D. (2007). *Foundation in Medical Mycology*, (1st ed.), Kenalf Publication, Port Harcourt Nigeria. P. 185-191.

Schewan, J.M. (1977). Microbiology of fish and fish product. Progress report. *Journal of Applied Bacteriology* 34: 299-315