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# MICROBIAL STATUS OF SMOKED FISH, *SCOMBIA SCOMBIA* SOLD IN OWERRI, IMO STATE, NIGERIA

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**ABSTRACT**

**As one of the common sources of protein available to man, fish is highly consumed due to its lower cholesterol content and price. So it forms a rich protein source for both poor and rich. As a part of checkmating the public health risks associated with this general dependence of the population on fish, the microbiological assessment of smoked fish, *Scombia, scombia* sold in Owerri was embarked on with the aim of ascertaining the microbial quality, the presence and prevalence of microorganisms of public health importance. A total of one hundred and eight (108) samples were collected from the smoking Factory, Open Market and Hawkers. These were analyzed microbiologically for viable heterotrophic bacteria and fungi count on Nutrient and Potato dextrose agar respectively, using pour plate method and coliform count in MacConkey broth by multiple tube method (MPN). The mean value results from the analysis revealed high microbial contamination in all the samples. The resultant data were analyzed statistically using randomized block design of Analysis of Variance (ANOVA) at 95% level of confidence and the difference were separated using the least significance difference (LSD). The mean results of viable heterotrophic bacteria and fungi count showed no significance difference for the collection sites; but the coliform mean results for the three sites showed marked variation at 95% level (P>0.05). Identified bacteria, include: *Staphylococcus aureus, E. coli, Bacillus sp., Klebsiella sp.*, whereas fungi are *Penicillium sp., Aspergilusl sp., Fusarium violaceum, Biospora sp., Candida sp, Botryodioplodia sp., Alternania sp.* This high level of microbial contamination can be traceable to handlers, and environment to which this fish is exposed during smoking and selling exercises, and considering the danger it portends to human health, public health and food safety authorities should intensify their monitoring efforts towards controlling such contamination.**

*Key words:* **Bacteria, Yeast, Mould, Smoked fish, Contamination.**

## INTRODUCTION

Fish is a vertebrate animal, living in fresh and seawater. It is one of the main sources of animal protein foods available for human consumption (**1**). Most of the catch comes from oceans, seas, rivers and lately from man-made ponds (**2**). It is a highly nutritious food of about 60-80% water, 15-25% protein, 11-22% fat, 20% mineral and 1% carbohydrate (**3**). It is often cheaper than meat and so it is a rich protein source for both the poor and the wealthy.

Microbial flora of fish depends on the microbial content of the water in which they live as the slime that covers the surface of fish has been found to contain great variety of bacteria genera (**4**). Many dangers therefore exist if fish harvested from polluted water is eaten raw, and because of the high microbial load of freshly harvested fish it is susceptible to rapid spoilage. Hence preservation of fresh fish becomes very important. This can be achieved by freezing, drying through smoking and sun-drying, canning, etc.

Smoking simply means a heating process that dries the fish to preserve it from spoilage (**5**). Most dry fish consumed in Nigeria are smoked (**6**). Smoking of fish from smoldering wood for its preservation dates back to civilization (**7**). The steps in the smoking process are necessary not only for safe preservation, but also to produce good flavor and aroma (**8**). Hence smoked fishes are less prone to microbial spoilage than fresh fish. However spoilage still occurs as a result of growth of microbes due to partial dehydration during smoking (**9**).

Contamination of fish and other fishery products by microbes has been a serious threat to human health. There are four main factors responsible for fish spoilage once it is out of its natural habitat (water) and these include: Autolysis which usually precedes bacterial spoilage and involves the breakdown of

protein and lipids to amino acids and fats by muscle enzymes. The activity of microorganism is another factor which uses the amino acid produced by autolysis for proliferation (**10)**. Others are chemical deterioration and insect attack which cause considerable deterioration.

However, spoilage of fresh and highly preserved fish products is mostly caused by microbial action. Foods of high sugar/salt contents are therefore most likely to be spoiled by any kind of microbe (**5)**. It has been reported that serious disease outbreak had occurred in both man and animals after consuming some dried fish feed and food (**11)**. This could be as a result of disease causing microorganism like *Escherichia coli, Salmonella typhi, Vibrio cholera* which results from poor handling/cross-contamination and improper processing practice of ready to eat "smoked fish" products. Other microorganisms of primary concern are *Listeria monocytogen* and *Clostridium botulinium*. Extensive handling provide opportunities for other food borne pathogens to contaminate products if sufficient attention is not given during smoking process (**12)**.

This work therefore tends to investigate the level of sanitation maintained by handlers during processing and storage of smoked fish sold in Owerri.

## MATERIALS AND METHODS

### Sample collection

A total of 108 smoked fish samples "*Scombia scombia*" were collected from the smoked fish factories, Open markets and Hawkers all within Owerri. Three weekly samples were collected for 3 Months between the months of February and April 2006. These were analyzed for microbial load at the Microbiology Laboratory of the Department of Medical Laboratory Science, Imo State University, Owerri.

### Sample preparation and Laboratory analysis

Ten (10) grams of the smoked fish was weighed into a stomacher bag and 90ml of sterile physiological saline was added. This was thoroughly homogenized in the stomacher for 90 second. Then ten-fold serial dilution was prepared in 9ml of solvent using 1ml sterile pipette. The viable heterotrophic bacterial counts, yeast and mould counts were done using pour plate method on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media respectively, while the Most Probable Number (MPN) of coliform was determined in MacConkey broth using Multiple Tube method.

### Pour Plate Method

An aliquot of 0.1ml from each dilution was aseptically transferred to the centre of sterile Petri-dishes in duplicates. Then sterile molten nutrient Agar/Potato Dextrose Agar at about $45^0$C was poured on them accordingly. These were mixed by a combination of rotational movement: To and fro, clockwise and anticlockwise direction for 5-10 seconds. The plates were allowed on the bench to solidify, inverted and properly labeled. These were incubated at $37^0$C for 24hrs and room temp for 3-7days for bacterial and fungal growths respectively.

### Most Probable Number (MPN) Method

Eleven test tubes with Durham's tubes inverted inside them were used. The tubes were labeled and grouped into three batches of one, five and five tubes for each batch. Batch one contained 50mls, batch two contained 10mls and batch three contained 5mls each. Each tube in the three batches was inoculated with 50mls, 10mls and 1ml of each sample respectively.
The inoculated tubes were corked and incubated at $44^0$C for 24hrs. Positive test tubes were indicated by colour change from red to yellow showing acid production and gas production was shown by the displacement of broth in the Durham's tube inside the tubes. Most Probable Number (MPN) of coliform was determined as stipulated by Chesbrough (2000) (**13)**.

The bacteria and fungi isolates were sub-cultured and preserved in Nutrient and potato Agar slants accordingly for further characterization. These were tested for Grams reaction, Motility and biochemical characteristics such as Catalase, Oxidase, Indole, Urease and Carbohydrate (sugar) utilization as stipulated by Baron *et al,* (1994) (**14)**.

### STATISTICAL ANALYSIS

The data obtained from this investigation were analyzed statistically using randomized block design ANOVA and the means separated using Least Significance Difference (LSD).

## RESULT

The result of microbial status of smoked fish, *Scombia scombia* fish sold in Owerri, Imo State are as follows: Microbial analysis was done to determine the general viable count, the coliform count as well as yeast and moulds count.

Table 1 shows the mean of viable heterotrophic bacteria count of smoked fish samples from the three sources and showed no significant difference at 95% level ($P<0.05$). Factory smoked fish recorded $8.83x10^5$ (cfu)/g, while market smoked fish recorded $1.35 x10^6$ (cfu)/g and Hawkers $2.50x10^6$ (cfu)/g.

Table 2 presents the Mean results of the coliform count which showed significant difference at 95% level ($P>0.05$). There were high coliform counts in all the samples. Factory smoked fish had mean count of $2.8 x 10^4$ (cfu)/g, Market smoked fish had $3.8x10^4$ (cfu)/g and Hawkers, $5.57x10^5$ (cfu)/g.

**TABLE 1: THE MEAN RESULT OF VIABLE HETEROTROPHIC BACTERIA COUNT OF SMOKED FISH SAMPLE (X10³ CFU/G)**

| Weekly Replication | smoked fish sources | | |
|---|---|---|---|
| Factory | Market | Hawkers | |
| 1 | 50 | 70 | 175 |
| 2 | 120 | 225 | 350 |
| 3 | 95 | 110 | 225 |
| Total | 265 | 405 | 750 |
| Mean | (88.3) | (135) | (250) |

F 0.05 (2, 6) = 5.14, P<5.14. *Not significant at 95%

**TABLE 2: THE MEAN RESULT OF COLIFORM COUNT OF SMOKED FISH (X10³ CFU/G), USING MOST PROBABLE NUMBER (MPN) METHOD.**

| Weekly Replication | smoked fish sources | | |
|---|---|---|---|
| Factory | Market | Hawkers | |
| 1 | 30 | 41 | 57 |
| 2 | 28 | 35 | 55 |
| 3 | 26 | 38 | 55 |
| Total | 84 | 114 | 167 |
| Mean | (28) | (38) | (55.7) |

F 0.05 (2,6) = 5.14, P>5.14. *Significant at 95%. LSD = 4.69

Table 3 displays mean values of fungal loads for the sites. The highest,0.2 x $10^4$ (cfu)/g was observed in samples from Hawkers, whereas samples from Factory and open market had the lowest,1 x$10^4$ (cfu) /g each. However there was no significant difference among the samples at 95% level (P>0.05).

**TABLE 3: FUNGAL COUNT (X10³CFU/G) FOR THE THREE SITES**

| Weekly Replication | Smoked fish sources | | |
|---|---|---|---|
| | Factory | Market | Hawkers |
| 1 | 1 | 1 | 3 |
| 2 | 1 | 1 | 2 |
| 3 | 1 | 1 | 1 |
| Total | 3 | 3 | 6 |
| Mean | (1) | (1) | (2) |

F 0.05 (2, 6) = 3.0, P>5.14. Not Significant at 95%

Table 4 shows sample location specificity for Bacteria isolates. This indicates that *Bacillus spp.* and *Staphylococcus aureus* were isolated from all the sources, while *E. coli* and *Klebsiella spp.* were isolated from Market and Hawkers respectively.

**TABLE 4: SPECIFIC SITES OF BACTERIAL CONTAMINANTS' ISOLATION.**

| Sources | No. of samples collected | No. contaminated | No of organisms | Bacterial Isolates isolated |
|---|---|---|---|---|
| **Factory** | **36** | **36** | **2** | ***Bacillus spp., Staph. aureus*** |
| **Market** | **36** | **36** | **3** | ***Bacillus spp., Staph. aureus,E. coli*** |
| **Hawkers** | **36** | **36** | **3** | ***Bacillus spp., Staph. aureus,Klebsiella spp.*** |

Table 5 shows fungal contaminants of smoked fish from the different sources. Fishes from Hawkers were more contaminated with *Candida spp., Fusarium spp.* and *Aspergellus spp.,* followed by those from market and factory which have *Alternaria spp.* and *Penicillum spp;* and (*Botryodioplodia spp* and *Biospora spp.,* respectively

**TABLE 5: SPECIFIC SAMPLES OF FUNGI CONTAMINANTS ISOLATION.**

| Sources | No. of samples collected | No. contaminated | No of organisms | Fungal Isolates isolated |
|---|---|---|---|---|
| **Factory** | **36** | **6** | **2** | ***Biospora spp, Botryodioplodia spp.*** |
| **Market** | **36** | **10** | **2** | ***Alternaria spp, Penicillum spp.*** |
| **Hawkers** | **36** | **20** | **3** | ***Candida spp, Fusarium spp, Aspergellum spp*** |

## DISCUSSION AND CONCLUSION

This work primarily aimed at investigating the maintenance of proper sanitary levels of processing and storage conditions by handlers of smoked fishes sold in Owerri. There were marked variations between the means of viable bacteria counts. Result showed high coliform contamination, compared with the standard ($10^3$). The high count, especially on the factory source could be attributed to improper pre/post handling /smoking procedures. This is in agreement with Maga, (1988) (15) who considered smoking process, a mild preservative treatment, which kills bacteria and prevents microbial proliferation due to combined effects off heating, drying, pH and Anti microbial smoke components. Hence, as a mild treatment, smoking does not achieve complete elimination of microbial load of a fresh fish which has been proved to be naturally high due to the high microbial load of their habitat (water) (4).

The highest counts observed among the samples from Hawkers can be attributed to the fact that hawking exposes the fish to more possibilities of contamination than any of the other sources. This supports the observation of Eklund *et al,* 1993 (12), which stated 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. Moreover, hawkers move from one place to and other hence, the possibility of exposing the fish to different microbial inhabitant of the different areas. This is unlike those of the factory and market that have limited exposition to microbial environments. Again hawkers are mostly children under the age of twelve. Who are not yet used to hygienic practices compared to their adult counterparts in the factory and some of the markets.

The isolation of *E. coli* and *Klebsiella spp.* are indications of feacal contamination and this is in agreement with the report of Frazier and Westhoff (1995) (4) which states that microbial flora of fish depends on the microbial contents of the waters in which they lived in. Dike*et al,2007*(16) has proven that water sources in Owerri, especially the streams and rivers from where these fishes were obtained are contaminated with coliform organisms. Hence the isolation of these feacal contaminant from fishes sold in Owerri is likely to be from those water sources.

Again the isolation of *Staphylococcus aureus and Bacillus spp.* is an indication of poor handling or cross contamination of smoked fish products, since the two organisms have been indicted in food poisoning (17). *Biospora spp., Botryodioplodia spp., Alternaria spp., Penicillum spp., Candida spp., Fusarium spp.,* and *Aspergellum spp.* as identified in this work have all been incriminated in food spoilage and are traceable to water and soil with which the fish is in contact (18). Also the isolation of these microorganisms from the smoked fish indicate partial dehydration during

smoking which is in agreement with Schewan (1977) (9) who attributed species of fungi observed in smoked fish samples to microbial spoilage as a result of partial dehydration during smoking.

The result of this work has proven that smoked fish sold in Owerri 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. This work also has shown that bacteria and fungi are responsible for the microbial contamination of smoked fish.

Based on these findings, we are recommending the use of mechanized smoking system that would completely dehydrate the fish in order to prevent contamination due to moisture. Also we are recommending that regulatory authority such as NAFDAC should look into the environmental condition of our food handlers as it concerns the smoking factories, the markets where our foods are sold and even the hawkers that carry the food from one place to another. Their hygienic condition must be ascertained before authorizing them to handle public food.

Finally, we are recommending that people should properly cook their fish before eating even when it is smoked to avert food poisoning.

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