Microbial Assessment and Health Risks of Consumption of Uncooked Smoked Horse Mackerel Fish (Trachurus trachurus) Sold in Open Markets in Owerri Metropolis, South Eastern Nigeria

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Aim: To assess the microbiological quality of uncooked smoked horse mackerel fish (Trachurus trachurus) sold in Owerri and ascertain the presence and prevalence of microorganisms of public health importance.

Study Design: Random sampling was done.

Place and Duration of Study: Department of Microbiology, Federal University of Technology, Owerri; from October, 2019 to April, 2020.

Methodology: Smoked fish samples (n=20) were purchased randomly from retailers in Relief, Naze, Eziobodo and Obinze markets and taken to the laboratory for isolation and identification of microorganisms. Standard microbiological methods were employed to analyze for viable heterotrophic bacterial and fungal counts on nutrient agar and potato dextrose agar respectively, using the spread and streak plate techniques. Coliform counts were done on MacConkey agar. Biochemical characterization of the microorganisms was adopted for their identification.
Results: Bacteria identified included *Staphylococcus, Escherichia, Proteus, Salmonella* and *Micrococcus* species. Total heterotrophic bacterial counts and coliform counts ranged from $2.8 \times 10^6$ cfu/g to $1.6 \times 10^8$ cfu/g and $2.7 \times 10^4$ cfu/g to $5.3 \times 10^5$ cfu/g respectively. Fungal species identified were *Penicillium, Rhizopus, Mucor, Aspergillus* and *Fusarium* spp and fungal counts as high as $4.5 \times 10^5$ cfu/g were recorded.

Conclusion: The high level of microbial contamination of the samples and the presence of organisms of public health importance signifies an obvious danger to human health. Eating smoked fish without proper cooking should be discouraged by the relevant authorities. Also, food safety authorities should intensify their monitoring efforts towards controlling such contaminations and averting possible outbreaks of diseases.

Keywords: Microbiological quality; smoked fish; spread plate; total heterotrophic bacterial counts; microbial contamination.

1. INTRODUCTION

Fish, a good protein source available for humans is safer and better than animal proteins because it contains lower cholesterol levels [1,2]. Reported that it is nutritious; containing water (60-80%), protein (15-25%), fat (11-22%), mineral (20%), and carbohydrate (1%). In Nigeria, animal proteins are now generally expensive as a result of poor technological advancement, limited pasture and climate, etc. and these have led to increased demand for fish-based protein [3]. In South Eastern Nigeria, fish is widely consumed just like in Niger Delta communities where fish is eaten more than meat [4].

Microbial contamination of fish and fish products has been a challenge to consumers all over the world. [5] in his work reported that the microbial flora of fish depends on the microbial quality of the water in which they live. Fish harvested from polluted water bodies poses a lot of health risks if not properly prepared for consumption. Due to the high microbial population of freshly harvested fish, it is susceptible to rapid spoilage. Hence, the need for the preservation of fresh fish by freezing, canning, smoking and sun-drying etc.

The smoking of fish for preservation dates back to early civilization [6]. Smoked fish is fish that has been cured by placing on fire from wood for drying [7,8]. Had earlier reported that smoking is not only for preservation but also to produce good flavor and aroma. According to [9], the aim of roasting fish is to minimize post-harvest losses and also enhance its shelf-life. This is as a result of the fact that the absence of water in the roasted fish reduces microbial growth in the fish sample which in turn reduces the rate of spoilage. However, as reported by [5], spoilage of fish still occurs as smoking does not totally dehydrate fish.

This work therefore was intended to determine the microbial quality and health risks associated with uncooked smoked fish sold in the Owerri metropolis.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Owerri metropolis, Imo State, South Eastern Nigeria. Owerri is the capital city and administrative headquarter of Imo State. Imo state is situated on latitude $5^\circ 29'N$ and $7^\circ 2'E$.

2.2 Test Media

The media used for this study are general purpose media (Nutrient agar), Mannitol Salt Agar (MSA), *Salmonella - Shigella* agar (SSA), Differential media (MacConkey agar) and Potato Dextrose Agar (PDA).

2.3 Collection of Samples

Twenty (20) uncooked smoked horse mackerel fish samples were purchased from fish retailers in Ihiagwa, Relief, Naze, Ezioibodo-FUTO road and Obinze markets, respectively and taken to the laboratory for isolation and identification of microorganisms. The analyses were done within 24hrs of collection.

2.4 Sterilization of Materials

The materials used for this study were sterilized using standard techniques as described by [1]. Glass wares were sterilized in the hot air oven at $160^\circ$C for 1 hour. Culture media were sterilized by autoclaving at $121^\circ$C 15psi pressure unit for 15 minutes. The inoculation wire loop was
sterilized by flaming intermittently to red hot over a Bunsen flame. Glass rod spreader (hockey stick) was sterilized intermittently by dipping in 70% alcohol and bringing it over a burning flame. Also Bench top, inoculation hood and working area were sterilized by disinfecting with Purit antiseptic. Sterile disposable hand gloves and face masks were worn and changed after each procedure to maintain aseptic conditions.

2.5 Processing of Test Samples

Each fish sample was processed for bacteriological and mycological analyses using standard microbiological techniques [10]. A sterile surgical blade was used to cut out one gram (1g) of each sample and placed inside a sterile 20ml test tube. The samples were serially diluted after maceration under aseptic conditions and appropriate dilutions were inoculated on the different agar media for total plate counts. Another 1g of the remaining fish sample was used for direct culture using the streaking method to isolate possible microorganisms. All cultures were incubated in duplicate at 37°C for 24 – 48 hours for bacterial counts and for 48-72 hours for fungal counts [10;11].

2.6 Preparation of Media and Microbiological Examination of Samples

The media used were Nutrient agar (N.A.), Salmomella Shigella Agar (SSA), Mackonkey Agar (MA), MannitoL Salt Agar(MSA) and Potato Dextrose Agar (PDA). All media used were prepared according to the manufacturers' instructions. The mean counts of bacteria in colony forming units per gram of samples were determined.

The samples were examined microbiologically for bacteria and fungi using standard culture techniques [10;11].

2.7 Identification of Bacterial and Fungal Isolates

The isolates were sub-cultured and identified using their cultural and morphological characteristics and biochemical tests. The bacterial isolates from the fish samples were identified using their cultural characteristics on different media used and biochemical identification tests. Gram staining, motility test, catalase, coagulase, oxidase, indole production,
citrate utilization, urease production, Hydrogen sulphide production, nitrate reduction, methyl red, VogesProskauer and sugar fermentation tests. Fungal solates were identified using macroscopic (cultural characteristics) and microscopic morphologies.

3. RESULTS AND DISCUSSION

Results as shown in Table 1 indicate that the Mean Bacterial Count (MBC) and Mean Fungal Count (MFC) for the Horse Mackerel fish sample from the different markets ranged from $2.8 \times 10^6$ to $1.6 \times 10^8$ cfu/g and $1.0 \times 10^4$ to $4.5 \times 10^5$ respectively. Salmonella was not detected in Obinze and Relief markets. Tables 2 and 3 represents the probable bacterial and fungal isolates from Horse mackerel.

The microbial analysis showed that the mean microbial counts of the fish samples were significantly higher than the permissible limit as stated by ICMSF. According to the International Commission on Microbiological Specifications for Foods (ICMSF), the acceptable counts of pathogenic organisms in food are; Staphylococcus sp ($<10^2$), Escherichia sp ($< 10^2$) and Salmonella sp ($<10^1$). There was no detectable colony of Salmonella sp at Obinze and Relief markets.

Most of the fish processors do not practice adequate hygiene, such as washing of hands after visiting the toilets or after touching cash notes. Moreover, storage after smoking is mostly under normal environmental temperature. The high counts could be attributed to improper pre/post handling and smoking procedures. This is in agreement with [5 and 13] who had earlier reported that smoking is a mild way of preservation which kills some bacteria and reduces microbial proliferation due to combined effects of heating, drying, pH changes and antimicrobial components of smoke. Consequently, as a mild preservation method, smoking does not completely eliminate microorganisms on fresh fish which has been proven to be naturally high because of the microbial quality of water, their natural habitat [5 and 14].

This study revealed the presence of Staphylococcus, Proteus, Salmonella, Micrococcus and Escherichia species on the uncooked smoked fish samples. Similar isolates have been recorded in previous studies by [1], [5], [15], [16] and [17]. The isolation of Escherichia and Salmonella spp. Indicates fecal contamination of the smoked fish samples. Furthermore, the isolation of Staphylococcus species indicates poor handling and/ or possibly cross contamination of the smoked fish products. The presence of Staphylococcus species in the smoked fish samples could have also arisen from dust in the environment due to the open display of fish products in the market.

Table 3 further revealed the presence of fungal species which include Mucor, Penicillium and Fusarium. These organisms had been earlier reported by [16] and [18] in previous studies with smoked fish samples. [19 and 20] found that these bacterial and fungal species are

| Market | Mean Fungal Count (cfu/g) | Mean Bacterial Count (cfu/g) | Mean Staphylococcal Count (cfu/g) | Mean Coliform Count (cfu/g) | Mean Salmonella Count (cfu/g) |
|--------|--------------------------|-----------------------------|----------------------------------|----------------------------|----------------------------|
| Naze   | 4.5 x 10^7               | 1.6 x 10^7                  | 4.85 x 10^6                      | 5.3 x 10^5                 | 4.6 x 10^3                 |
| Ihiagwa| 2.0 x 10^4               | 2.8 x 10^5                  | 1.4 x 10^5                      | 4.2 x 10^4                 | 2.7 x 10^5                 |
| Obinze | 1.0 x 10^5               | 7.5 x 10^5                  | 1.5 x 10^5                      | 2.7 x 10^4                 | Ni                        |
| Relief | 2.5 x 10^6               | 4.1 x 10^5                  | 3.6 x 10^5                      | 2.7 x 10^6                 | Ni                        |

Key: cfu/g = Colony forming units per gram; Ni = Not isolated

| Market | Bacterial species Isolates                  |
|--------|---------------------------------------------|
| Naze   | Escherichia, Staphylococcus, Proteus, Salmonella |
| Ihiagwa| Staphylococcus, Salmonella Micrococcus,     |
| Obinze | Staphylococcus, Escherichia                 |
| Relief | Staphylococcus Micrococcus,                 |
Table 3. Probable fungal isolates of horse mackerel fish

| Market   | Fungal species Isolates       |
|----------|-------------------------------|
| Naze     | Fusarium, Aspergillus         |
| Ihiagwa  | Penicillium                   |
| Obinze   | Fusarium, Aspergillus         |
| Relief   | Mucor, Rhizopus               |

associated with fish spoilage and possibly food poisoning. *Penicillium* and *Fusarium* spp. have been implicated severally in smoked fish spoilage and as reported by [21] could be attributed to water and soil which comes in contact with the fish.

The high microbial counts and presence of some possible pathogenic organisms suggests that smoked fish when not adequately cooked poses serious public health risks to consumers, such as Salmonellosis (caused by *Salmonella* species), gastroenteritis (by *Salmonella* and *Escherichia* species), Staphylococcal food poisoning and mycotoxicosis (by *Fusarium*) etc [19].

4. CONCLUSION

The result of this work has shown that uncooked smoked horse mackerel fish sold in open markets in Owerri is contaminated. This study further reveals the presence of possible pathogenic microorganisms in the smoked fish samples as well as high bacterial and fungal counts. Smoking is therefore not an effective method of total prevention of microbial contamination of fish and the presence of these microorganisms in the smoked fish is a public health risk, especially in immuno-compromised individuals.

5. RECOMMENDATION

It is important and very necessary to properly cook smoked fish before eating to ensure its safety and handlers of smoked fish during processing should be properly trained to apply good hygiene practices in order to minimize contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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