MICRO-ORGANISMS ASSOCIATED WITH SMOKED PRAWN (*Macrobranchium* spp.) IN SELECTED MARKET LOCATIONS IN ABEOKUTA METROPOLIS OF Ogun State, Nigeria

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ABSTRACT

This study is aimed at isolating and characterizing micro-organisms of *Macrobranchium* spp. gotten from markets in Abeokuta. Twelve different samples of whole smoked prawns (*Macrobranchium* spp.) were purchased from two different locations each in six major markets (Itoku, Omida, Iberekodo, Lafenwa, Panseke and Olomore) within the Abeokuta metropolis in Ogun State. Their microbial load was analyzed using Mac-Conkey agar (MA), Deoxycholate citrate agar (DCA), Nutrient agar (NA), and Mannitol salt agar (MSA) for bacteria isolation while Potato Dextrose agar (PDA) was used to isolate the fungi in the microbiology laboratory of the department of Microbiology, Federal University of Agriculture, Abeokuta. *Staphylococcus aureus* and *Citrobacter* spp (22.22% each) dominated the samples while the fungal specie that occurred most frequently in the samples was *Aspergillus niger* (31.03%). The total bacterial counts for all the samples ranged from $9 \times 10^2$ to $1.0 \times 10^3$ cfu/g and fungal count were between 21%-90% in terms of frequency of occurrence. These microorganisms cause food spoilage and poisoning and the occurrence of such microorganisms may be as a result unhygienic handling of prawns during processing as some of the microorganisms may be post-harvest contaminants. Adequate cooking could help in reducing microorganism of smoked prawn.

Keywords: Bacteria, fungi, microbial load, smoked prawn, spoilage.

INTRODUCTION

Food, fish and other aquatic product's insecurity in developing and under developed countries has led to the evasion of some diseases attributable to the consumption of these products. Seafood however refers to all fresh or salt water organisms such as shellfishes, fin fishes, mollusks, crustaceans and all other forms of aquatic animal life. Prawn, a source of animal protein is one of the commonest in Africa and Asia countries. The short supply of animal protein in Nigeria to a level almost beyond the reach of the low income earners has also made this group of seafood an alternative source of animal protein. Prawns are low in fat and calories, contain a lot of omega-3 fatty acids, a high level of vitamin B$_{12}$, zinc, iodine, phosphorus, potassium, selenium and iron but have smaller quantity of magnesium, calcium and sodium (Food and Drug Admini-
In order to prevent spoilage and increase shelf life of fresh prawn, value adding through further processing (e.g. smoking) is necessary. Smoking is one of the traditional fish processing methods aimed at preventing or reducing post-harvest losses. Unfortunately, much of the prawn smoked today is exposed to smoke just long enough to provide the desired flavor with little if any drying (Chinivasagama et al., 1996; Piggot, 2009), making the prawns susceptible to spoilage. Processing of a fish species and shellfish inevitably entails a storage period for the finished product prior to marketing and consumption. Since fish are composed of perishable nutrients, storage period should be kept to a minimum with adequate storage conditions provided so as to prevent deteriorative changes occurring through oxidative damage and/or microbial, insect or rodent infestation. The most important environmental factors governing the storage or shelf life of fish are ambient temperature and humidity. These factors dictate the rate at which chemical changes take place (Daramola et al., 2007). In the present research an attempt has been made to investigate the microorganisms associated with smoked prawn (Macrobranchium spp.) in selected market locations in Abeokuta metropolis of Ogun State.

MATERIALS AND METHODS

The Study Area

The study was carried out in Abeokuta metropolis, comprising of Abeokuta North and Abeokuta South Local Government Areas of Ogun State in Nigeria. Good majorities of the people in these local government areas are farmers, traders and civil servants as well as non-farm workers. Abeokuta metropolis has a hot humid climate with annual rainfall of about 1200mm. It is in the tropical rain forest zone of Nigeria. The rural farming population estimated for Abeokuta metropolis as at 1993 was 34,262 and this gives a projected figure of 55,431 by the year 2010, given the population growth rate of 2.83 percent (OGADEP, 1993; FGN, 1997; FMA, 1997). The study area is part of Abeokuta zone as classified by Ogun State Agricultural Development Programme (OGADEP). The state has a total water surface area of 2,237,000 hectares (Ita et al., 1984) and land area of 16,369,370 square kilometers. The Abeokuta zone of unified extension services was purposively selected due to the fact that fish farming business are majorly embarked upon by the people in the zone (Olaoye et al., 2007).

Sampling Procedures and Sample size

Ready-to-eat smoked dried prawns were purchased from 6 different markets; Lafenwa, Olomore, Kuto, Itoku, Iberekodo and Panseke. A total number of 12 samples were collected [2 from each location] and kept in cellophane bags and transported to the laboratory. The samples collected included the carapace, entails and the exoskeleton of the prawn (Macrobranchium spp.).

Preparation of sample for culture

Ten (10) grammes of the whole prawn sample for microbiological evaluation were weighed into 9ml of sterile distilled tap water in the bijou bottle. This was done for samples gotten from each location and was taken as the original stocks sample of each market.
location. 1ml of the original stocks solution was poured into 9ml sterile distilled water and mixed thoroughly to give \(10^{-2}\) of the original sample making a tenfold serial dilution.

**Isolation and characterization of bacterial isolates**

Samples were inoculated on Mac-Conkey agar (MA), Deoxycholate citrate agar (DCA), Nutrient agar (NA) for isolation of organisms, Mannitol salt agar (MSA) for selective isolation of *Staphylococcus* and Potato Dextrose agar (PDA) for isolation of fungi. Inoculated plates were incubated aerobically for 24 hours at 37°C while fungal culture plates containing acidified PDA were incubated at 25°C for 3-7 days. Colony counts were done using digital unlimited colony counter and counts expressed in coliform forming unit per gram (cfu/g) of the sample. The bacteria colonies were sub cultured on fresh media and identification was done using standard procedure and biochemical tests such as gram staining, catalase test, citrate utilization test, sugar fermentation test, coagulase test, indole test and oxidase test (Fawole and Oso, 2001, Jimoh et al., 2009, Olutiola et al., 1991, Taylor and Stone, 2008).

**Isolation of fungi**

The samples were inoculated on PDA and incubated at room temperature for 3 days. After incubation, plates were sub cultured on freshly prepared PDA agar to get pure culture of the organisms (Ibrahim and Rahma, 2009).

**Identification of fungi**

This was done according to James and Natalie (2001) using cotton blue in lactophenol stain. The identification was done by placing a drop of the stain on a clean slide with the aid of a mounting needle; a small portion of the mycelium from the cultures was removed and placed in a drop of lactophenol. The mycelium was spread on the slide using a needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide the mounted and viewed under x10 and x40 objective lenses respectively. The species observed were identified according to Cheesbrough (2000), Ibrahim and Rahma, (2009).

**Data Analysis**

The data from this study were analysed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) and further presented using tables, percentages and figures.

**RESULTS AND DISCUSSION**

The total count (in CFU/g) of bacteria and fungi present in the ready-to-eat prawns is given in Table 1. The total counts were generally high and it ranged from \(1.1 \times 10^3\) to \(9 \times 10^2\) cfu/g. Samples from locations 1 and 3 (Kuto and Lafenwa) possessed the highest bacteria load (22.04% and 22.95%) respectively; locations 2 and 5 (Iberekodo and Olomore) had the least bacteria load of 10.63% & 9.56% respectively.

Though, location 3 had a higher bacterial load as compared with location 1 but there was no significant difference between the bacterial loads of the samples from the two locations. Similarly, location 3 (Lafenwa) had the highest load of fungi (32.89%), followed by samples from location 2 (Iberekodo 22.09%), samples from 4 had the least fungi load.
Table 1: Microbial load on prawn samples (cfu/g)

<table>
<thead>
<tr>
<th>Sample code</th>
<th>NA</th>
<th>MSA</th>
<th>MA</th>
<th>PDA</th>
<th>Suspected organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1A</td>
<td>2.14X10^4</td>
<td>2.13X10^4</td>
<td>3.40X10^3</td>
<td>2.14X10^4</td>
<td>S.aureus, P. aeruginosa, Shigella spp, Citrobacter spp, A. niger, Penicillium spp, C. krusei, A. oryzae</td>
</tr>
<tr>
<td>S1B</td>
<td>2.15X10^4</td>
<td>2.14X10^4</td>
<td>3.3X10^3</td>
<td>2.16X10^4</td>
<td>S.aureus, P. aeruginosa, Shigella spp, Citrobacter spp, A. niger, Penicillium spp, C. krusei, A. oryzae</td>
</tr>
<tr>
<td>S2A</td>
<td>8.00X10^2</td>
<td>7.00X10^2</td>
<td>9.00X10^2</td>
<td>1.20X10^3</td>
<td>S.aureus, B. cereus, Proteus vulgaris, E. coli, Citrobacter spp, A. niger, Mucor spp</td>
</tr>
<tr>
<td>S2B</td>
<td>8.00X10^2</td>
<td>8.00X10^2</td>
<td>8.00X10^2</td>
<td>1.1 X 10^3</td>
<td>S.aureus, B. cereus, Proteus vulgaris, E. coli, Citrobacter spp, A. niger, Mucor spp</td>
</tr>
<tr>
<td>S3A</td>
<td>2.42X10^4</td>
<td>2.39X10^4</td>
<td>3.00X10^2</td>
<td>2.64X10^4</td>
<td>S.aureus, Penicillium spp, E. coli, Citrobacter spp, A. niger, Mucor spp</td>
</tr>
<tr>
<td>S3B</td>
<td>1.21X10^4</td>
<td>2.43X10^4</td>
<td>2.9X10^3</td>
<td>2.68X10^4</td>
<td>S.aureus, P. aeruginosa, E. coli, Citrobacter spp, A. niger, Mucor spp</td>
</tr>
<tr>
<td>S4A</td>
<td>1.21X10^4</td>
<td>1.24X10^4</td>
<td>2.00X10^2</td>
<td>2.82X10^4</td>
<td>S.aureus, Citrobacter spp, A. niger, Seratia spp, Pseudomonas spp, Penicillium spp</td>
</tr>
<tr>
<td>S4B</td>
<td>1.20X10^4</td>
<td>1.22X10^4</td>
<td>2.00X10^2</td>
<td>2.96X10^4</td>
<td>S.aureus, Citrobacter spp, A. niger, Seratia spp, Pseudomonas spp, Penicillium spp</td>
</tr>
<tr>
<td>S5A</td>
<td>7.00X10^2</td>
<td>7.00X10^2</td>
<td>8.00X10^2</td>
<td>9.00X10^2</td>
<td>S.aureus, Citrobacter spp, A. niger, Seratia spp, A flavus, Proteus vulgaris</td>
</tr>
<tr>
<td>S5B</td>
<td>8.00X10^2</td>
<td>7.00X10^2</td>
<td>7.00X10^2</td>
<td>9.00X10^2</td>
<td>S.aureus, Citrobacter spp, A. niger, Seratia spp, A. oryzae, Mucor spp, Pseudomonas spp</td>
</tr>
<tr>
<td>S6A</td>
<td>2.14X10^3</td>
<td>3.00X10^3</td>
<td>2.18X10^4</td>
<td>3.10X10^3</td>
<td>S.aureus, Citrobacter spp, A. niger, Seratia spp, A. oryzae, Penicillium spp, C. krusei</td>
</tr>
<tr>
<td>S6B</td>
<td>2.42X10^4</td>
<td>2.14X10^4</td>
<td>2.43X10^4</td>
<td>3.10X10^3</td>
<td>S.aureus, Citrobacter spp, A. niger, Seratia spp, A. oryzae, Penicillium spp, C. krusei</td>
</tr>
</tbody>
</table>

Though, location 3 had a higher bacterial load as compared with location 1 but there was no significant difference between the bacterial loads of the samples from the two locations. Similarly, location 3 (Lafenwa) had the highest load of fungi (32.89%), followed by samples from location 2 (Iberekodo 22.09%), samples from 4 had the least fungi load.

Bacteria from nine different genera were isolated and identified from the prawn samples obtained from the 6 market locations in Abeokuta (Tables 1 & 2). Staphylococcus aureus and Citrobacter spp (Figure 1) dominated the samples (22.22% each) this is a confirmation of the work of (Okonta and Ekelemu, 2005) who reported Staphylococcus as one of the predominant micro-organisms affecting smoked fish and causing their spoilage. Bacillus cereus (3.70%) occurred least in the samples. Bacillus is the normal microbial flora of the fish and are not initially harmful, as they help in preventing the invasion of the fish flesh by other micro-organisms but may become pathogenic as a result of poor handling, poor hygiene and delayed processing and preservation of the prawn after harvest, other bacteria species occurred sparingly. The presence of Staphylococci is usually indicative of contamination from the skin, mouth or nose of food handlers (Jimoh et al., 2009). Inadequately cleaned equipment or raw animal products may also be a source of contamination. The presence of large numbers of bacteria in the collected samples in the study areas was a good indication of poor hygiene and temperature control.

The presence of substantial numbers of E. coli in foods suggest a general lack of cleanliness in handling and improper storage. Bacilli spp. was only isolated from prawn samples from locations 2 and 6. It is a gram-positive, obligate aerobe rod shaped, endospore forming bacteria (Todar, 2008). Two Bacillus spp. are considered medically significant; B. anthracis which causes anthrax and B. coagulase also causes food spoilage. Colonially, they are large, spreading and irregularly shaped. When viewed under microscope, they appear as rods with a bulge which contains the endospore (Martinko, 2005).

Among the fungal isolates, Aspergillus niger had the highest percentage of occurrence of 31.03%, while Rhizopus specie had the least percentage of occurrence of 6.90% (Figure 2). Other fungi family: Penicillium (20.69%), Aspergillus oryzae (13.79%), Aspergillus flavus (6.90%), Mucor spp. (20.69%) occurred sparingly in the samples. Only one class of yeast (Candida krusei) was isolated and identified from the samples. The presence of microorganisms in these samples might be the result of contamination during sales or unhygienic handling of the prawns by the sellers who display the products in the open market places without covering them. In a similar study conducted by (Edema and Agbon, 2010), on the significance of fungi associated with smoked-cured Ethmalosa fimbriata and Clarias gariepinus, it was observed that the nutrient value of these smoked cured fish is not significantly diminished by the smoking process but that the economic value may be determined by the quality of the fish presented for sale.

Location 3 (Lafenwa) had the highest load of fungi (32.89%), followed by samples from location 2 (Iberekodo: 22.09%), then samples from 4 had the least fungi load. It was also observed in this study that the presence of fungi particularly aflatoxigenic moulds in these fish species is very significant from a food safety point.
Table 2: Morphological, biochemical and tentative identification of bacteria from prawn samples

<table>
<thead>
<tr>
<th>Gram reaction</th>
<th>Citrate utilization test</th>
<th>Catalase test</th>
<th>Coagulase test</th>
<th>Motility test</th>
<th>Indole test</th>
<th>Glucose</th>
<th>Lactose</th>
<th>Sorbose</th>
<th>Suspected organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Shigella spp</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td>Citrobacter spp</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AG</td>
<td>+</td>
<td>+</td>
<td>Klebsiella spp</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>AG</td>
<td>-</td>
<td>-</td>
<td>Proteus vulgaris</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>AG</td>
<td>-</td>
<td>AG</td>
<td>Serratia spp</td>
</tr>
</tbody>
</table>

Figure 1: Percentages of occurrence of bacteria spp. present in the prawn samples
Table 3: Cultural and morphological characteristics of fungi isolated from the prawn samples

<table>
<thead>
<tr>
<th>Shape</th>
<th>Surface</th>
<th>Elevation</th>
<th>Spore colour</th>
<th>Type of mycelium</th>
<th>Type of reproduction</th>
<th>Septation</th>
<th>Appearance of special structures</th>
<th>Suspected microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filamentous</td>
<td>Powdery</td>
<td>Raised</td>
<td>Black</td>
<td>Conidiospore</td>
<td>Sexual</td>
<td>Septate</td>
<td>Foot cells</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>Circular</td>
<td>Fluff</td>
<td>Raised</td>
<td>Greenish Blue</td>
<td>Conidiospore</td>
<td>Sexual</td>
<td>Septate</td>
<td>-</td>
<td>Penicillium spp</td>
</tr>
<tr>
<td>Circular</td>
<td>Powdery</td>
<td>Semi-Raised</td>
<td>Black</td>
<td>Conidiospore</td>
<td>Sexual</td>
<td>Septate</td>
<td>-</td>
<td>Aspergillus oryzae</td>
</tr>
<tr>
<td>Filamentous</td>
<td>Woven Mass</td>
<td>Raised</td>
<td>Black</td>
<td>Sporogiospore</td>
<td>Asexual</td>
<td>Non-septate</td>
<td>Rhizoid</td>
<td>Rhizopus spp</td>
</tr>
</tbody>
</table>

Figure 2: Percentage of occurrence of fungi species present in the prawn samples
CONCLUSION

In conclusion, Prawns which serves as an alternative source of animal protein cannot be banned from consumption based on its nutritional values; hence efforts should be made by relevant organizations on the formulation and enforcement of laws which would promote proper post-harvest technologies and good hygiene practice of prawns and other seafood products. Also, efforts should be geared towards awareness programs amongst food vendors about safe and hygienic practices and its importance to the health of man. Consumers also need to recognize that a healthy food means a healthy heart hence, prawns and other foods purchased from open markets should be processed further through washing and heating.

RECOMMENDATIONS

It is hereby recommended that
i. Smoked prawn packaging should be fly-proof in order to prevent the invasion of microbial growth on the products;
ii. Proper handling, processing and preservation of the prawns and other sea foods must be ensured; and
Consumers of smoked prawns and other sea foods should clean wash and subject these products to further cooking or heating so as to destroy all heat labile microorganisms present.

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