Full Length Research Paper

Microbiological assessment of traditional smoked silver catfish (Chrysichthys nigrodigitatus)

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Received 23 September, 2014; Accepted 12 December, 2014

Smoked fish is relished food item in many dishes in Nigeria. Traditional smoked silver catfish (Chrysichthys nigrodigitatus) that floods the smoked fish market of the Lagos State of Nigeria are not microbiologically shelf-stable; hence, the need for a study on their microbiological quality and safety. ‘Fresh silver catfish’ (100 samples) and smoked silver catfish (100 samples) were collected from 20 different processing centres and the fresh samples were smoked with convectional smoke kiln as control and microbiological analyses were conducted. Each batch was assessed for: total viable count (TVC), fungal count (FC), Listeria monocytogenes (LM) count, Staphylococcus aureus (SA) count, Salmonella Paratyphi (SP) count and presence or absence of Escherichia coli (EC). The results obtained showed significant variations (p<0.05) for all the microbial counts of the smoked fish samples. The mean TVC range of 6.6 x 10⁸ - 8.8 x 10⁹ cfu/g was recorded for fresh silver catfish samples and 2.0 x 10¹ - 8.6 x 10⁴ and 1.0 x 10³ - 5.4 x 10⁵ cfu/g for samples of smoked silver catfish. 5.0 x 10⁵ - 6.4 x 10⁷ cfu/g was recorded for fresh silver catfish samples and from 5.6 x 10¹ - 60.4 x 10² cfu/g and 1.0 x 10⁵ - 3.5 x 10⁵ cfu/g for samples of smoked silver catfish (SA). Mean (LM) of fresh silver catfish samples was 1.8 x 10² - 2.5 x 10² cfu/g and 1.3 x 10¹ - 13.2 x 10¹ cfu/g for samples of smoked silver catfish. Mean (FC) of smoked silver catfish from local drum kiln was 4.1 x 10¹ - 8.2 x 10¹ cfu/g. The samples of smoked silver catfish using conventional smoke kiln showed no count for L. monocytogenes, S. Paratyphi and E. coli.

Key words: Silver catfish, smoking, traditional, Staphylococcal, Listeria monocytogenes.

INTRODUCTION

Fish is an important dietary component of people all around the world and represents a relatively cheap and accessible source of high quality protein for poorer households (Ikutegbe and Sikoki, 2014). In West Africa, fish has been reported to provide 40–70% of the protein intake of the population (Béné and Heck, 2005; Ikutegbe and Sikoki, 2014) and is a critical source of dietary protein that is not readily available in the carbohydrate-based staple foods of the population. Depending on consumer preference, there are several forms in which fish can be consumed; fresh, dried, frozen, fermented, brined, etc. In a study by Mafimisebi (2012), it was discovered that majority of the Nigerian people reported a preference for fresh fish; however limitations such as the

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low keeping quality of the fish after harvest and the distances between fishing grounds and marketing outlets make this very difficult. This results in a higher reported consumption of smoke-dried fish, which has a longer shelf-life (Mafimisebi, 2012). In Nigeria, fish has an edge over meat because it is cheaper and relatively more abundant (Eyo, 2001) and constitutes about 40% of the animal protein intake (Eyo, 2001; Abolagba and Melle, 2008).

Because fish is highly perishable, a considerable effort has been directed to extend its shelf-life using preservation and processing techniques, such as refrigeration, freezing, canning, smoking, salting and drying (Nwachukwu and Madubuko, 2013). Besides this, some of these techniques can also be used to enhance the value of fish, such as smoked fish.

Smoked fish is relished food item in many dishes in Nigeria. The technique has developed to a point where once common food has become a delicacy and there is need for corresponding concern for safety issues in smoked fish consumption (Riches, 2012). Da Silva et al. (2008) examined the microbial safety and quality of smoked blue catfish (Ictalurus furcatus) steaks treated with antimicrobials and antioxidants during 6 weeks ambient storage. Fafioye et al. (2002) studied the fungal infestation of five traditionally smoked dried freshwater fish in Agolo-Iwoye, Nigeria, and isolated and identified eleven different fungal species of which Aspergillus flavus was the most frequently encountered fungi on the fish species. Adebayo-Tayo et al. (2008) reported the presence of aflatoxin and other metabolites in smoked fish due to A. flavus in smoked fish sold in Uyo, Akwa Ibom State, Nigeria and confirmed that consumers could have been at risk of aflatoxin poison.

According to Aberoumand (2010), Escherichia coli is a classic example of enteric bacteria causing gastroenteritis. E. coli including other coliforms and bacteria such as Staphylococcus spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. Scientists have shown that the contamination of food of fish origin with pathogenic E. coli probably occur during handling of fish and during the production process (Jimoh et al., 2009). The microorganisms associated with smoked fish pose a great threat to the populace as the transfer of the microorganisms attack the immune system of the consumer, usually man, thereby, giving room for the invasion of disease. E. coli and Staphylococcus aureus were reported as the predominant microorganisms present in smoked fish in Asaba area of Delta State of Nigeria (Okonta and Ekelemu, 2005). Outbreak of Listeriosis in different parts of the world in the last three decades as a result of eating smoked fish has been a major public health concern.

This study is therefore, embarked on to assess the microbiological quality of traditional smoked catfish (Chrysichthys nigrodigitatus) and by so doing, identify bacterial and fungal species prevalent in traditional smoked catfish, their distribution, effects and possible public health implications of the presence of such microorganisms.

**METHODOLOGY**

**Fish used**

Fresh fish samples were obtained from ten different fishing communities of Badagry and Epe Local Government Areas of Lagos State, Nigeria. The fresh silver catfish samples were freshly harvested. The samples were taken to the IFSERAR laboratory, Federal University of Agriculture, Abeokuta for smoking.

**Area of study**

Using a current geopolitical map of Nigeria (Figure 1), Lagos State lies to the south-western part of Nigeria with 20 local government areas (LGAs) and has boundaries with Ogun State both in the north and east. It is bordered on the west by the Republic of Benin and in the south, stretches for 180 km along the coast of the Atlantic Ocean. It therefore has 22.5% of Nigeria’s coastline and occupies an area of 3,577 sq km land mass with about 786.94 sq km (22%) of it being lagoons and creeks. The state is endowed with marine, brackish and fresh water ecological zones with varying fish species that provide productive fishing opportunity for fishermen. Two local government areas (Badagry and Epe Local Government) were covered because they are highly dense fish processing centers. They were selected for the study and hazard analyses of the products.

**Sampling procedure**

Fresh silver catfish (100 samples) and smoked silver catfish (100 samples) were collected from 10 processing centres from each of the two local government areas by purposive sampling in sterile containers (Ziploc).

All freshly harvested silver catfish samples were kept on ice during transportation to the laboratory and smoked on the same day. Smoked fish samples were analyzed immediately.

**Fish smoking process**

Smoked fish was prepared following the method as described by Crapo (2011) with modifications. Fish were carefully cleaned to remove slime, blood and harmful bacteria. The fish were eviscerated, leaving the skin on the fish. The fish were cut into uniform pieces (fillet) so that no parts will get overheated. The fish were smoked to 80°C internal temperature (with a thermometer) for at least 24 h. The kiln temperature was adjusted as needed throughout this smoking period to maintain the 80°C internal temperature. Hands, utensils and work surfaces were cleaned when transferring fish from smoker to oven to cool down to avoid cross-contamination. Smoking was done for 24 h until the fish was fully dried.

**Physico-chemical analysis**

Kent pH meter (Kent Ind. Measurement Ltd., survey) model 7020 equipped with a glass electrode was used to measure the pH of the flesh, employing 10 g of fish homogenized in 10 ml of distilled water. Triplicate determinations were made in all cases. The pH
meter was calibrated using pH 4.0 and 7.0 buffers. All chemicals used in this study were of the analytical grade unless stated otherwise.

Microbiological studies

The presence of pathogens in fresh and smoked fish samples was investigated. These include: *Listeria monocytogenes*, *Salmonella Paratyphi*, *E. coli*, *S. aureus* and fungal count. Fish samples (fresh and smoked) obtained from the identified processing centres were analyzed microbiologically. The microbiological procedures recommended in the International Commission on Microbiological Specification for Foods (ICMSF, 1986) were applied. Culture media were those of Oxoid, Biolife and Difco. For each sample, 25 g were weighed out and transferred to a sterile blender with 225 ml of 0.1% peptone and mixed thoroughly for 2 min to prepare fish homogenate. These were then analyzed as follows.

Total viable bacterial counts

Appropriate dilutions of the fish homogenate were prepared and inoculated onto sterile Petri dishes. Plate count agar (Oxoid) media were then poured. Plates were incubated at 35–37°C for 48 h and colonies were then counted and reported as total colony count/ml. A second set of plates was incubated at 35–37°C for 48 h in a carbon dioxide incubator or under anaerobic conditions using a gas pack anaerobic jar. Colonies were then counted and reported as anaerobic total bacterial count. In case of spore formers count, the food homogenate was boiled first at 75–80°C and then rapidly cooled. Appropriate serial dilutions were prepared and inoculated onto the surface of sterile and dried plate count agar media. These were incubated finally at 35–37°C for 48 h.

Detection of *Escherichia coli*

1 ml of each of the decimal dilutions of the fresh and smoked fish homogenate was plated on poured Eosine Methylene Blue Agar (Oxoid) and then incubated at 35–37°C for 24 h. Counts were calculated from the number of growth on the plates. The colonies with green metallic sheen were counted as *E. coli*.

Detection of *S. aureus*

A sample of 0.1 ml of the fresh and smoked fish homogenate and dilutions was inoculated on Baird-Parker (Difco) agar plates and incubated at 35–37°C for 48 h. Colonies appearing to be black and shiny with narrow white margins and surrounded by clear zones were identified by coagulase test reactions. The coagulase test was carried out by first inoculating typical colonies in brain heart infusion broth (Difco) and incubating at 37°C for 24 h. From the resulting cultures, 0.1 ml was then added to 0.3 ml of rabbit plasma in sterile tubes and incubated at 37°C for 4 h. The formation of a distinct clot was evidence of coagulase activity.

Detection of *Salmonella Paratyphi*

Samples of fresh and smoked fish homogenate and dilutions were inoculated in Salmonella-shigella agar (Oxoid) and incubated at 35–37°C for 24 h. For identification, 2–3 suspected colonies were inoculated into tryptone broth for indole test, triple sugar iron agar slant (Oxoid), urea broth and lysine iron agar. These were incubated at 37°C for 24 h. *Salmonella* species is indole negative, on triple sugar iron it produces acid (yellow) and alkaline (red) with or without gas and hydrogen sulfide, is urea negative, and on lysine iron agar shows an alkaline (purple) reaction throughout the medium. Serological tests were then carried out.

Detection of *L. monocytogenes*

A sample of 0.1 ml of the fresh and smoked fish homogenate and dilutions was inoculated on Brilliant Listeria Agar (Oxoid) plates and incubated at 35–37°C for 24 h. Colonies appearing were counted and reported as *L. monocytogenes*.
counted and reported as fungal count/ml. Were incubated at 22–25°C for 3 days and then colonies were
cooling system in their boats or canoes. However, the pH
from the sea because most of the fishermen had no
biochemical reactions and enzyme action as a result of delay in reaching the shore
high, this may be due to biochemical reactions and
fish tissues drops due to smoking (Doe, 1998; da Silva,
enzyme action as a result of delay in reaching the shore
was high, this may be due to biochemical reactions and
Enzyme action as a result of delay in reaching the shore
was high, this may be due to biochemical reactions and
is the most critical factors affecting microbial growth
and spollage of foods. The pH value of fresh silver catfish
samples (Table 1), ranged from 6.90 – 7.24 and pH value
of smoked silver catfish samples ranged from 6.27 – 6.86
and 6.5 – 6.86. The pH values of the fresh fish samples
was high, this may be due to biochemical reactions and
was high, this may be due to biochemical reactions and
was high, this may be due to biochemical reactions and
was high, this may be due to biochemical reactions and
for L. monocytogenes for fish and fishery products is the presence of the organism, that is zero tolerance so most of the samples from local drum kiln do not meet the ICMSF recommended microbial specification. Therefore, the smoked silver catfish samples from all processing centres need to be cooked before consumption in order to destroy L. monocytogenes that is present in the smoked silver catfish to prevent possibility of food poison by listeriosis. All the smoked silver catfish samples of convention smoke kiln tested negative for L. monocytogenes while the fresh fish samples contained L. monocytogenes. Goktepe and Moody (1998) reported that Listeria spp. counts of raw catfish fillets were 4.37 log CFU/g; after brining, the count decreased slightly to 3.24 log CFU/g and no Listeria spp. was detected in for L. monocytogenes for fish and fishery products samples after hot smoking. Staphylococcal count of fresh silver catfish samples (Table 1) ranged from 5.0 x 10² - 6.4 x 10³ cfu/g and that of samples of smoked silver catfish from different processing centres (local drum kiln) ranged from 5.6 x 10² - 60.4 x 10² cfu/g and 1.0 x 10² – 3.5 x 10² cfu/g (Table 2). The Staphylococcal count values obtained for the smoked silver catfish were below the specified recommended value for all fish. The S. aureus safety level is equal to or greater than 10⁶/g and in many cases, these levels represent the point at or above which the agency will take legal action to remove products from the market (FDA, 2001). In addition, smoking also reduced Staphylococci and fungal counts. The isolation of S. aureus in smoked samples can be attributed to post processing contamination. S. Paratyphi was not detected in smoked silver catfish samples obtained using local drum kiln and

Table 2. Microbial quality (cfu/g) and pH of smoked silver catfish (Chrysichthys nigrodigitatus) from 20 different processing centres using local drum kiln and conventional smoke kiln.

<table>
<thead>
<tr>
<th>Locations</th>
<th>Listeria Monocytogenes</th>
<th>Salmonella Paratyphi</th>
<th>E. coli</th>
<th>Staphylococcal count</th>
<th>Fungal count</th>
<th>T.V.C.</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agbalata</td>
<td>4.0 x 10¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23.4 x 10⁶</td>
<td>4.6 x 10⁴</td>
<td>6.43abcd</td>
</tr>
<tr>
<td>Ajido</td>
<td>1.4 x 10²</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17.1 x 10⁶</td>
<td>6.1 x 10⁴</td>
<td>6.48bcdef</td>
</tr>
<tr>
<td>Asakpo</td>
<td>6.1 x 10²</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>39.0 x 10⁵</td>
<td>5.4 x 10⁵</td>
<td>1.0 x 10⁵</td>
</tr>
<tr>
<td>Boguru</td>
<td>7.5 x 10⁴</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60.2 x 10⁴</td>
<td>5.0 x 10⁵</td>
<td>1.1 x 10⁵</td>
</tr>
<tr>
<td>Fvanoveh</td>
<td>11.0 x 10⁴</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.5 x 10⁴</td>
<td>2.5 x 10⁶</td>
<td>1.8 x 10⁶</td>
</tr>
<tr>
<td>Gberefun</td>
<td>1.4 x 10¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18.6 x 10⁵</td>
<td>2.6 x 10⁶</td>
<td>1.0 x 10⁵</td>
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<tr>
<td>Ilaje</td>
<td>16.0 x 10⁴</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.0 x 10⁴</td>
<td>1.0 x 10⁵</td>
<td>1.2 x 10⁵</td>
</tr>
<tr>
<td>Kofegameh</td>
<td>12.3 x 10⁴</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>34.2 x 10⁴</td>
<td>5.5 x 10⁵</td>
<td>1.3 x 10⁵</td>
</tr>
<tr>
<td>Pako</td>
<td>2.6 x 10¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>19.5 x 10⁵</td>
<td>3.3 x 10⁵</td>
<td>1.1 x 10⁵</td>
</tr>
<tr>
<td>Afuye</td>
<td>2.5 x 10¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.6 x 10⁵</td>
<td>2.4 x 10⁵</td>
<td>1.0 x 10⁵</td>
</tr>
<tr>
<td>BodinYawa</td>
<td>4.1 x 10¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18.0 x 10⁵</td>
<td>4.1 x 10⁵</td>
<td>3.5 x 10⁵</td>
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<tr>
<td>Idale</td>
<td>1.3 x 10⁴</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18.1 x 10⁵</td>
<td>4.1 x 10⁶</td>
<td>1.4 x 10⁶</td>
</tr>
<tr>
<td>Igbobun</td>
<td>5.1 x 10¹</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>56.5 x 10⁴</td>
<td>8.0 x 10⁴</td>
<td>1.2 x 10⁴</td>
</tr>
<tr>
<td>Ilogun</td>
<td>7.0 x 10⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>39.2 x 10⁴</td>
<td>3.1 x 10⁴</td>
<td>1.2 x 10⁴</td>
</tr>
<tr>
<td>Mejona</td>
<td>8.5 x 10⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45.0 x 10⁴</td>
<td>4.1 x 10⁴</td>
<td>1.2 x 10⁴</td>
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<tr>
<td>Oluwo</td>
<td>6.0 x 10⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60.4 x 10⁴</td>
<td>8.0 x 10⁴</td>
<td>1.2 x 10⁴</td>
</tr>
<tr>
<td>Okorisa</td>
<td>13.2 x 10⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.1 x 10⁴</td>
<td>2.2 x 10⁴</td>
<td>1.4 x 10⁴</td>
</tr>
<tr>
<td>Orita</td>
<td>16.0 x 10⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.2 x 10⁴</td>
<td>4.5 x 10⁴</td>
<td>2.0 x 10⁴</td>
</tr>
<tr>
<td>Orogono</td>
<td>11.4 x 10⁰</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.5 x 10⁴</td>
<td>5.1 x 10⁴</td>
<td>1.4 x 10⁴</td>
</tr>
</tbody>
</table>

Data are means of 3 replicates. Data with different subscripts in the same column indicate significant difference at p<0.05. T.V.C = total viable count; - = no count.
conventional smoke kiln and this conformed with the specified microbiological limits recommended by ICMSF (1986) for *S. paratyphi* count for fish and fishery products which is the presence of the organism, that is, zero tolerance. In all cases, this suggests Good Manufacturing Practices (GMP) and no faecal contamination of the products as *Salmonella paratyphi* and *E. coli* serve as indicator organisms for faecal contamination of foods. In this study, fungal count of samples of smoked silver catfish from different processing centres (local drum kiln) ranged from $1.1 \times 10^1$ - $10.0 \times 10^1$ CFU/g (Table 2). The populations of fungi in the samples were all below $5 \times 10^5$ CFU/g specified microbiological limits recommended by ICMSF (1986) for fungi, except for the samples from convention smoke kiln that had no fungi count.

**Conclusion**

The pH values of the fresh fish samples were found to be high. However, the pH in fish tissues drops due to smoking. From this study, smoking significantly (p<0.05) reduced the pH and total viable count in all samples of smoked silver catfish using local drum kiln; however, the samples of smoked fish using conventional smoke kiln showed no count for *L. monocytogenes*, *S. paratyphi* and *E. coli* were not detected in all smoked silver catfish samples obtained using local drum kiln and conventional smoke kiln and this conformed with the specified microbiological limits recommended by ICMSF (1986) for *S. paratyphi* and *E. coli* count for fish and fishery products which is the presence of the organisms, that is, zero tolerance. In all cases, this suggests GMP and no faecal contamination of the products as *S. paratyphi* and *E. coli* serve as indicator organisms for faecal contamination of foods.

**Conflict of interests**

The authors did not declare any conflict of interest.

**REFERENCES**


