131 Hygiene Status of Fish Smoking Facilities of Selected Fish Smoking Centers in Ibadan, Southwestern Nigeria

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Abstract
The hygiene status of fish smoking facilities of selected fish smoking centers in Ibadan metropolies Oyo state South-Western Nigeria was studied. 45 swab samples were collected at 3 different sampling points (storage material, washing material and smoking kiln) from 5 different fish smoking centers in the study area while 15 smoked fish samples (Scomber japonicus) and water samples each were also collected. The microbial analysis was done using standard microbiological procedures. Most of the fish smoking facilities within Ibadan metropolies have poor hygiene status as their storage materials, washing materials, water samples, smoking kilns and smoked fish products have Total Coliform Counts (TCC), Feacal Coliform Counts (FCC) and Salmonella/Shigella counts above recommended level. Lowest level of TPC was obtained from smoked fish samples from Elebu (4.004 ± 0.01), Aleshinloye (4.301± 0.01), Mapo (4.230±0.04) and Moor Plantation (4.205±0.02) respectively significantly (P<0.05) lower than samples from other sampling points. Similar trend was observed for Total Fungal Counts (TFC), TCC, FCC and Salmonella/Shigella counts in most of the fish smoking centers. It is recommended that fish processors should get good water source for cleaning their fish and facilities as poor water source could aid transmition of microorganisms and diseases within fish processing facilities and this will have negative effect the smoked fish product. The need for fish processors to improve their sanitary conditions was encouraged. It was also recommended that consumers should properly wash smoked fish with clean portable water before eating so as to reduce the risk of food born diseases.

Key words: hygiene, status, fish smoking facilities, smoking centers

Introduction
Fish is an important source of good quality protein required in human diets, it has higher level of easily metabolisable protein, fat, vitamins, calcium, iron, and essential amino acids when compared to other sources of animal protein such as poultry and beef. (Ayeloja *et al.,* 2011). Fishery products constitute an important part of international trade, currently worth more than US$ 50 billion, indicating increasing consumer interest in the commodity (Alberth *et al.,* 2003). A major goal for the food processing industries is to provide safe, wholesome and acceptable food to the consumer and control of microorganisms is essential to meet this objective (Baggen-Ravn *et al.* 2003). However, this can be very difficult as contamination of products in a food processing environment may take place at all stages, during production (both pre and post-harvesting) and processing (Alberth *et al.,* 2013). Most commonly reported food preparation practices that contribute to foodborne diseases by scientist include; poor environmental hygiene, inadequate cooking, contaminated equipment, improper holding temperatures and food from unsafe sources (Baluka *et al.*, 2015). Havelaar *et al.* (2010) stated that measurement of the safety of foods relied on evaluations of the microbiological quality of foods. Nkere *et al.* (2011) also stated that bacteriological counts in prepared food or water is a key factor in assessing the quality and safety of food and can reveal the hygiene level adopted by food handlers in the course of food preparation. Outbreaks of foodborne illnesses have been linked to improper food handling practices at food serving establishments (Hassan *et al.*, 2010). However, there is lack of information on hygiene status of fish smoking facilities in this study area thus the need for this research which is aimed at determining hygiene indicators of fish processing facilities in selected fish smoking centers within Ibadan metropolies in order to identify their sources and routes of microbial transmission so that they could be reduced or eliminated.

Materials and Method
Study area - The study was carried out in Ibadan metropolies of Oyo state South-Western Nigeria. Where a sample each of a smoked fish were collected from five locations within Ibadan metropolies which includes; Omi-Adio, Elebu, Aleshinloye, Mapo, and Moor Plantation.

Sample Collection
A total of forty five swab samples containing normal saline were collected in triplicates from three different sampling points (storage material, washing material and smoking kiln) at five different fish smoking centers (Omi-Adio, Elebu, Aleshinloye, Mapo, and Moor Plantation fish smoking centers). The Smoked fish (*Scomber japonicus*) and water samples were collected from these smoking centers in triplicates (totaling 15 fish and water samples each). The Smoked fish (*Scomber japonicus*) were collected and kept in sterile polythene bags while the water samples were collected using sterile water sampling bottles then transported to the laboratory for microbial analysis with minimal delay.

Laboratory Analysis

Media preparation

The work areas were disinfected using swab with ethanol and all glass wares were sterilized in hot air oven at 160°C for 1hour. Culture media used for the total plate counts was Plate Count agar; MacConkey agar was used for *coliform counts;* feacal coliforms count was carried using Eosin Methylene Blue agar, Samonella and Shigella were counted using Salmonella-shigella agar while fungi colonies were counted using the potato dextrose agar. All culture media were prepared according to manufacturers' instructions and was sterilized in an autoclave at 121°C for 15minutes before use.

Microbial Analysis

Sample preparation, inoculation and incubation - 10g of each smoked fish samples were weighed aseptically and homogenized in 90ml sterile peptone water. Smoked fish samples were collected from the smoking centers were minced after which serial dilution was carried out. The storage material, washing material and smoking kiln and water samples collected were also serially diluted. One ml of the serially diluted samples (10^{-2} and 10^{-3} diluent) were inoculated into sterile petri dishes containing prepared culture medias in triplicates by pour plate method then allowed to solidified and incubated at 37°C for 24hours. Sub-culturing of colonies - Colonies on Plate Count Agar (PCA), Eosin Methylene Blue (EMB), MacConkey, Salmonella-shigella and Potatoes Dextrose agars were counted using the colony counter. The distinct colonies were sub-cultured on nutrient agar and incubated for 24hours at 37°C. Characterization and identification of the isolates - Bacterial isolates were identified based on morphological (shape, elevation, pigmentation and odour) and biochemical characterization described by Olutiola *et al.* (1991); Fawole and Osho (1995) after which they were identified using Bergey's Manual of Determinative Bacteriology.

Gram staining - A thin smear of each bacterial isolate was made and heat-fixed. The heat-fixed smears was covered with crystal violet which is a secondary staining for about 1 minute and rinsed with clean water. Then flooded with iodine as a mordant for 1 minute and then rinse immediately. The isolates were decolorized for 10 – 30seconds using 95% ethyl alcohol (destaining). Alcohol action was terminated by rinsing the slide with clean water, then the smear was counter-stained with safranin for 30seconds and then it was rinsed off using clean water and after which it was allowed to air dry. The stained slides were examined under the microscope at X100 (with the aid of immersion oil) for results. Gram-positive organisms appeared in purple while gram-negative organisms appeared in pink or red.

Motility test - This was carried out using hanging drop method according to Fawole and Osho (1995). Clean grease free depression slide and cover slides was used for the motility test, then a very little amount of Vaseline was placed near each corner of the cover slide. Two loopful of the suspended isolate were picked using a sterile wire loop placed on the depression slide and covered with the cover glass. The slide was quickly inverted and examined under the microscope, and then the motions of the organisms were observed.

Biochemical Test - Some of the biochemical test carried out include: catalase test, indole test, citrate test, sugar fermentation test, methyl red and oxidase test etc.

Statistical Analysis

Data obtained from the counted colonies were logarithmically transformed (log cfu/g). The data were subjected to Analysis of Variance (ANOVA) while means of the significantly different indices were separated using Duncan Multiple Range Test (DMRT) at $p < 0.05$.

Results

The result presented on table 1 indicates that there is no significant difference ($p > 0.05$) in the TPC of the various samples collected from different points in Omi-Adio fish smoking centre, these counts ranges from 4.102 ± 0.01 to 4.748 ± 0.01. However, there were significant differences ($p < 0.05$) in the TFC, TCC FCC and Salmonella/Shigella counts in the samples collected from Omi-Adio fish smoking centre. The highest TFC (4.643 ± 0.01) was obtained from storage material, while the highest TCC and FCC which are 4.812 ± 0.02 and 4.812 ± 0.02 respectively were obtained from the samples collected from fish smoking kiln. The water sample from this smoking centre had the highest Salmonella/shigella counts 4.799 ± 4.01. However, Smoked fish sample from this centre had the lowest TFC and Salmonella/shigella counts (4.100 ± 0.01 and 4.361 ± 0.03 respectively) while there was no growth (NG) for TCC and FCC in the fish sampled from this facility.

Table 1: Microbial Load of different sampling points and fish sample at Omi-Adio fish smoking center

	Sampling point	Total plate count (log Cfu/g)	Total fungal counts (log Cfu/g)	Total coliform counts (log Cfu/g)	Feacal coliform counts (log Cfu/g)	Salmonella/Shigella counts (log Cfu/g)
1	Stor. material	4.102 ± 0.01^{a}	4.643 ± 0.01^{c}	4.631 ± 0.01^{c}	4.631 ± 0.01^{b}	4.477 ± 0.01^{b}
2	Wash. material	4.102 ± 0.01^{a}	4.126 ± 0.01^{b}	4.126 ± 0.01^{a}	4.100 ± 0.01^{a}	4.672 ± 0.01^{c}
3	Smoking kiln	4.748 ± 0.01^{a}	4.105 ± 0.01^{d}	4.812 ± 0.02^{d}	4.812 ± 0.02^{b}	4.748 ± 0.01^{d}
4	Water sample	4.103 ± 0.01^{a}	4.102 ± 0.01^{a}	4.533 ± 0.01^{b}	4.103 ± 0.01^{a}	4.799 ± 4.01^{e}
5	Smoked fish	4.102 ± 0.01^{a}	4.100 ± 0.01^{a}	NG	NG	4.361 ± 0.03^{a}

Values with different superscript in the column indicates significant difference at $P < 0.05$; NG: No growth

There are significant differences ($P < 0.05$) in the TPC, TFC, TCC, FCC and Salmonella/shigella counts in the samples collected from Elebu fish smoking centre (Table 2). The result indicates that the highest TPC (4.724 ± 0.01) was obtained from water

sample at this centre while the highest TFC (4.818 ± 0.03) was obtained from the smoking kiln. The highest TCC (4.643± 0.01) was obtained from washing material, while the highest FCC (4.825 ± 0.02) was obtained from smoking kiln, and the water sample from this smoking centre had the highest Salmonella/shigella counts (4.806±0.01). However, no growth of TFC, FCC and Salmonella/shigella counts was observed on the smoked fish samples from this centre, it also had the lowest TPC (4.004 ± 0.01).

Table 2: Microbial Load of different sampling points and fish sample at Elebu fish smoking center

	Sampling point	Total plate count (log Cfu/g)	Total fungal counts (log Cfu/g)	Total coliform counts (log Cfu/g)	Feacal coliform counts (log Cfu/g)	Salmonella/Shigella counts (log Cfu/g)
1	Stor. material	4.301 ± 0.01^{b}	4.100 ± 0.01^{a}	4.217 ± 0.01^{a}	4.631 ± 0.01^{a}	4.107 ± 0.01^{a}
2	Wash. material	4.538 ± 0.01^{d}	4.102 ± 0.01^{a}	4.643 ± 0.01^{d}	4.806 ± 0.01^{b}	4.102 ± 0.01^{a}
3	Smoking kiln	4.447 ± 0.01^{c}	4.818 ± 0.03^{b}	4.273 ± 0.01^{b}	4.825 ± 0.02^{b}	4.124 ± 0.03^{a}
4	Water sample	4.724 ± 0.01^{e}	NG	4.494 ± 0.03^{c}	4.538 ± 0.14^{a}	4.806 ± 0.01^{b}
5	Smoked fish	4.004 ± 0.01^{a}	NG	4.777 ± 0.03^{e}	NG	NG

Values with different superscript in the column indicates significant difference at $P < 0.05$; NG: No growth

The result of samples collected at Aleshinloye fish smoking center (Table 3) indicates that there are significant differences ($P< 0.05$) in the TPC, TFC, TCC, FCC, and salmonella/shigella counts of samples collected from Aleshinloye fish smoking centre. However, no growth of Salmonella/shigella counts was observed on the smoked fish samples from Aleshinloye fish smoking centre, it also had the lowest TPC (4.301 ± 0.01) as well as low TFC (4.1328 ± 0.01) and FCC (4.100 ± 0.01) while samples collected from fish smoking kiln had the highest TPC and Salmonella/shigella counts (4.756 ± 0.01 and 4.843 ± 0.03 respectively).

Table 3: Microbial Load of different sampling points and fish sample at Aleshinloye fish smoking center

	Sampling point	Total plate count (log Cfu/g)	Total fungal counts (log Cfu/g)	Total coliform counts (log Cfu/g)	Feacal coliform counts (log Cfu/g)	Salmonella/Shigell a counts (log Cfu/g)
1	Storage material	4.574 ± 0.01^{c}	4.284 ± 0.02^{b}	4.004 ± 0.01^{a}	4.204 ± 0.02^{a}	4.276 ± 0.01^{a}
2	Washing material	4.491 ± 0.02^{b}	4.812 ± 0.02^{c}	4.175 ± 0.04^{b}	4.451 ± 0.09^{b}	4.806 ± 0.01^{b}
3	Smoking kiln	4.756 ± 0.01^{d}	4.100 ± 0.01^{a}	4.089 ± 0.12^{ab}	4.131 ± 0.01^{a}	4.843 ± 0.03^{c}
4	Water sample	4.863 ± 0.01^{e}	4.806 ± 0.01^{c}	4.216 ± 0.06^{b}	4.126 ± 0.01^{a}	4.284 ± 0.01^{a}
5	Smoked fish	4.301 ± 0.01^{a}	4.1328 ± 0.01^{a}	4.640 ± 0.01^{c}	4.100 ± 0.01^{a}	NG

Values with different superscript in the column indicates significant difference at $P < 0.05$; NG: No growth

Table 4 shows the result obtained on samples from Mapo fish smoking center and this indicates that there are significant differences ($P<0.05$) in the TPC, TFC, TCC, FCC, and salmonella/shigella counts in the samples collected from different points at Mapo fish smoking centre. At this fish smoking centre, smoked fish samples collected had the lowest TPC (4.230 ± 0.04), TFC (4.130 ± 0.04) and TCC (4.132 ± 0.05) while no growth of salmonella/shigella was observed. However, water sample had high TPC (4.942 ± 0.04), TCC (4.672 ± 0.01) and FCC (4.342 ± 0.03) at this centre.

Table 4: Microbial load of different sampling points and fish sample at Mapo fish smoking center

	Sampling point	Total plate count (log Cfu/g)	Total fungal counts (log Cfu/g)	Total coliform counts (log Cfu/g)	Feacal coliform counts (log Cfu/g)	Salmonella/Shigella counts (log Cfu/g)
1	Storage material	4.427 ± 0.01^{b}	4.251 ± 0.03^{a}	4.175 ± 0.04^{a}	4.004 ± 0.01^{a}	4.856 ± 0.04^{c}
2	Washing material	4.589 ± 0.02^{c}	4.869 ± 0.04^{b}	4.361 ± 0.03^{b}	4.079 ± 0.01^{ab}	4.844 ± 0.06^{c}
3	Smoking kiln	5.009 ± 0.04^{d}	4.633 ± 0.04^{ab}	4.491 ± 0.02^{c}	4.371 ± 0.01^{c}	4.321 ± 0.03^{b}
4	Water sample	4.942 ± 0.04^{d}	4.464 ± 0.49^{ab}	4.672 ± 0.01^{d}	4.342 ± 0.03^{c}	4.134 ± 0.01^{a}
5	Smoked fish	4.230 ± 0.04^{a}	4.130 ± 0.04^{a}	4.132 ± 0.05^{a}	4.150 ± 0.07^{b}	NG

Values with different superscript in the column indicates significant difference at $P < 0.05$; NG: No growth

The result on Table 5 indicates that there is no significant difference ($P>0.05$) in the Salmonella/shigella of the storage material and water samples collected from this centre and no growth of Salmonella/Shigella was observed on fish samples, washing material and smoking kiln at Moor plantation fish smoking centre. Also, fish sample from this centre had no fungal growth and low TPC (4.205 ± 0.02), TCC (4.240 ± 0.04) and FCC (4.144 ± 0.05) while the storage material at this centre had high TPC (4.532 ± 0.02), TCC (4.576 ± 0.09) and FCC (4.777 ± 0.03).

Table 5: Microbial Load of different sampling points and fish sample at Moor Plantation fish smoking center

	Sampling point	Total plate count (log Cfu/g)	Total fungal counts (log Cfu/g)	Total coliform counts (log Cfu/g)	Feacal coliform counts (log Cfu/g)	Salmonella/Shigella counts (log Cfu/g)
1	Storage material	4.532 ± 0.02^{c}	4.293 ± 0.03^{b}	4.576 ± 0.09^{c}	4.777 ± 0.03^{c}	4.154 ± 0.03^{a}
2	Washing material	4.431 ± 0.02^{b}	4.812 ± 0.05^{d}	4.538 ± 0.02^{c}	4.118 ± 0.01^{a}	NG
3	Smoking kiln	4.491 ± 0.02^{c}	4.683 ± 0.05^{c}	4.167 ± 0.01^{a}	4.477 ± 0.05^{b}	NG
4	Water sample	4.431 ± 0.02^{b}	4.124 ± 0.03^{a}	4.341 ± 0.20^{b}	4.126 ± 0.04^{a}	4.135 ± 0.05^{a}
5	Smoked fish	4.205 ± 0.02^{a}	NG	4.240 ± 0.04^{ab}	4.144 ± 0.05^{a}	NG

Values with different superscript in the column indicates significant difference at $P < 0.05$; NG: No growth

Discussion

The result of this study indicates that Lowest level of TPC was obtained from smoked fish samples from most of the fish smoking centers including; Elebu (4.004 ± 0.01), Aleshinloye (4.301± 0.01), Mapo (4.230±0.04) and Moor Plantation (4.205±0.02) respectively significantly ($P<0.05$) lower than samples from other sampling points. Similar trend was observed for Total Fungal Counts (TFC), TCC, FCC and Salmonella/Shigella counts in most of the fish smoking centers. This low microbial load on smoked fish could be as a result of smoke and heat applied during the fish smoking process. This is corroborated by Dillon *et al.* (1994) who stated that smoking usually extends the shelf life of fish due to the reduced moisture content due to heat applied and the effects of imparted phenolic compounds resulting in direct microbial destruction. Ndakatu *et al.* (2011) also stated that the effect of heat and dryness associated with the hot smoking reduces the water activity of the fish thereby limiting microorganisms, a prerequisite for spoilage. Ray *et al.* (2004) also stated that the steps involved in fish smoking involves heating and cooking the fish thereby reducing microbial load on smoked fish. Ayeloja *et al.* (2011) also reported that smoking prolongs fish shelf-life and enhances fish flavor. The result of this study also indicates that microbial load including total coliform counts, feacal coliform counts and Salmonella/Shigella counts were present in all the water samples used for cleaning fish at the onset of the fish processing exercise indicating that contamination of smoked fish do take place in the processing centers, water to be used for fish cleaning being a vehicle for the transmission of many agents of diseases (Kirby *et al*. 2003) must be clean and of good quality so as to avoid further contamination of the raw fish which will in turn affect the end product. Although, contamination of raw fish with high pathogens do occur before they are brought to fish processing units, this has been reported by many scientist like Huss *et al*. (2003) who pointed out that some pathogenic bacteria are naturally present in the aquatic (*Clostridium botulinum* type E, pathogenic *Vibrio* sp., *Aeromonas*) and the general environment (*C. botulinum*, type A and B, *Listeria monocytogenes*) of fish and are found on live or raw fish. Venugopal (2002) also reported that contamination of fish particularly by pathogens such as *Salmonella* sp., *Staphylococcus aureus*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, and *Listeria monocytogenes*, do occur prior to harvest, during capture, processing, distribution and storage. Contaminated water in this case is most likely to have contributed to increase microbial load of other fish processing facilities and affect the smoked fish product. This corroborates the opinion of Alberth *et al.* (2003) who stated that water, unhygienic activities in food processing operations and unhygienic food processing environment are the main source of food contamination. Montville *et al*. (2002) also reported that, during handling and preparation, bacteria may be transferred from contaminated hands of food workers to food and subsequently to other surfaces (including food contact surfaces). Dike-Ndudim *et al.* (2014) also stated that high coliform contamination in fish smoking facility above the standard (10^3) can be attributed to improper hygiene. The result obtained in this study is similar to that reported by Udochukwu *et al.* (2016) that higher bacteria isolates were observed from swab samples collected from the Uselu and New Benin market compared to that Oba market in Edo State, Nigeria while fungal isolates from Oba was higher than those of Uselu and New Benin market. Values obtained for fungal counts in this study was lesser than those reported for Benin city by Udochukwu *et al.* (2016) in their study of comparative assessment of the microbiological quality of smoked and fresh fish sold in Benin city and its public health impact on consumers. The result of this study indicates that the sanitary conditions of fish smoking kiln in most of the fish smoking facilities within Ibadan metropololies is very poor expect Moor Plantation fish smoking centre compared with others where the sanitary indicators such as faecal coliform counts, total coliform counts and Salmonella/Shigella counts in most of the other facilities were beyond the recommended level which 3 $\log_{10}$ Cfu/g (*Dike-Ndudim et al., 2014).* Christianah *et al*. (2010) attributed poor hygiene, particularly deficient or absence of hand washing as the causative mode of transmission. It is therefore important to apply proper cleaning and sanitation procedures so as to increase the quality of smoked produced within Ibadan metropolies which will in turn have positive health implication on the consumers and the society at because a healthy nation is a wealthy nation.

Conclusion

This study indicates that most of the fish smoking facilities within Ibadan metropolies have poor hygiene status as their storage materials, washing materials, water samples, smoking kilns and smoked fish products have Total Coliform Counts (TCC), Feacal Coliform Counts (FCC) and Salmonella/Shigella counts above recommended level. Lowest level of TPC was obtained from smoked fish samples from Elebu (4.004 ± 0.01), Aleshinloye (4.301± 0.01), Mapo (4.230±0.04) and Moor Plantation (4.205±0.02) respectively significantly ($P<0.05$) lower than samples from other sampling points. Similar trend was observed for Total Fungal Counts (TFC), TCC, FCC and Salmonella/Shigella counts in most of the fish smoking centers. However, fish processing facility of moor plantation Ibadan had the best hygiene status when compared to others but the hygiene condition of its storage facilities needed to be improved upon. Fish processors should get a good water source for cleaning their fish and facilities as poor water source could increase microbial load of other fish processing facilities and affect their smoked fish

product. Fish processors also need to improve their sanitary conditions as their environment could also serve as source of fish contamination, proper application of hygiene and sanitation procedures in fish processing facilities within Ibadan metropolis should be improved and monitored by relevant agency of Government. In addition, proper washing of smoked fish with portable water should be adopted before eating so as to reduce the risk of food born diseases associated with smoked fish consumption because a healthy nation is a wealthy nation.

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