American Journal of Microbiological Research, 2016, Vol. 4, No. 1, 37-40
Available online at http://pubs.sciepub.com/ajmr/4/1/4

DOI:10.12691/ajmr-4-1-4

Comparative Assessment of the Microbiological Quality of Smoked and Fresh Fish Sold in Benin City and Its Public Health Impact on Consumers

U. Udochukwu[1,*], J. Inetianbor[2], S.O Akaba[3], F.O. Omorotionmwan[3]

[1]Department of Biosciences, Salem University-Lokoja, Kogi State, Nigeria
[2]Department of Microbiology, Federal university Wukari, Taraba State, Nigeria
[3]Department of Microbiology, University of Benin, Edo State, Nigeria
*Corresponding author: rev.dr.ud@gmail.com

Abstract This study assessed the microbiological qualities of smoked and fresh fishes sold in Benin Metropolis. The microbial counts of fresh fish samples were comparably higher than those of smoked fish samples. For the smoked fish, the count ranged from 8.344 x 10^5 – 3.108 x 10^6 for bacterial count and 1.693 x 10^5 – 9.043 x 10^5 cfu/g for fungal count, while for the fresh fish, it ranged from 2.774 x 10^6 – 4.416 x 10^6 for bacterial count and 5.787 x 10^5 – 1.840 x 10^6 cfu/g for fungal count. Specific bacteria isolated were *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Acinetobacter* spp., *Streptococcus pyogenes, Klebsiella* spp., *Micrococcus luteus, Enterobacter aerogenes, Flavobacterium* spp., *Corynebacterium* spp., *Serratia marcescens* and *Staphylococcus epidermidis.* The fungal isolates were *Penicillium expansum, Saccharomyces* spp., *Aspergillus niger, Fusarium* spp., *Rhizopus stolonifer* and *Mucor piriformis.* Also, this study revealed that moisture content and pH influenced the microbial load of fish. The moisture content and pH values of smoked fish ranged from 12.62±0.14 – 33.91±0.34 and 5.87±0.06 – 6.63±0.02 respectively. For the fresh fish samples, moisture content and pH values ranged from 52.95±0.64 – 63.30±0.13 and 6.57±0.003 - 6.83±0.003 respectively. From this study, it was observed that fish prepared under hygienic conditions had lower microbial load. Statistically, t-test showed that there is a difference in the microbial load of smoked and fresh fish. It was observed that the shelf life, method of preservation and handling processed could affect the microbial load and diversity. Therefore, the adoption of a good processing practice, the use of controlled temperature in processing and preserving of fish products and proper storage of both smoked and fresh fish are highly recommended.

Keywords: *microbiological quality, smoked and fresh fishes, bacteria, fungi, public health impact*

Cite This Article: U. Udochukwu, J. Inetianbor, S.O Akaba, and F.O. Omorotionmwan, "Comparative Assessment of the Microbiological Quality of Smoked and Fresh Fish Sold in Benin City and Its Public Health Impact on Consumers." *American Journal of Microbiological Research*, vol. 4, no. 1 (2016): 37-40. doi: 10.12691/ajmr-4-1-4.

1. Introduction

Fish are classified as any of the cold-blooded aquatic vertebrates of the super class Pisces typically showing gills, fins and a streamline body. In addition, fish also refers to any other form of marine or fresh water animal that can be used for human consumption [1]. Fish is an important part of a healthy diet since it contains high quality protein but typically presents a low fat percent when compared to other meats. Fish can be eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers [2]. Preserving food and other perishable products like fish and meat generally involves processes that impede growth of microorganisms either by addition of growth inhibiting agents or adjusting storage conditions by freezing or drying. Smoking is the preferred microbiologically safe method of fish preservation compared to fresh fish which have a heavy pathogenic microbial load. In preserving fish by smoking, water activity in the fish is lowered to the point where the activity of spoilage microorganisms is inhibited and the wood smoke add some microbial inhibitory substances like formaldehyde and alcohols [3]. Therefore fish requires proper handling and preservation to increase its shelf life and retain its quality and nutritional attributes. Immediately a fish is caught, it loses its natural resistance to attack by microorganisms and also starts to undergo both physical and chemical changes that in return bring changes in appearance, taste, smell and texture.

The autochthonous bacterial flora of fish is dominated by Gram-negative genera including: *Acinetobacter, Flavobacterium, Moraxella, Shewanella* and *Pseudomonas*. Members of the families *Vibrionaceae* (*Vibrio* and *Photobacterium*) and the *Aeromonadaceae* (*Aeromonas* spp.) are also common aquatic bacteria, and typical of the fish flora. Gram-positive organisms such as *Bacillus, Micrococcus, Clostridium, Lactobacillus* and *Coryneforms*

can also be found in varying proportions [4]. Human pathogenic bacteria can be part of the initial microflora of fish, posing a concern for sea foodborne illnesses: especially organisms naturally present on fish. Fish and shellfish poisonings are due to toxins in the flesh of the fish either intrinsically present or derived from the food they eat. This is true for puffer fish poisoning (caused by tetradotoxin), ciguatera (caused by ciguatoxin and possibly other toxins) and paralytic shellfish poisoning (PSP) (caused by saxitoxin and other toxins). Fish and shellfish products are a minor source of bacterial foodborne disease in North America, the United Kingdom, and Australia [5] but there is a continuing high relative incidence of bacterial foodborne disease from fish products in Japan and probably in Southeast Asian countries where fish are commonly eaten raw [6]. A number of different *Vibrio* species have been implicated in foodborne illness resulting from eating seafoods. These include *V. parahaemolyticus, V. vulnificus, V. cholerae, V. fluvialis* and *V. alginolyticus.* Disease due to *Vibrio* species is usually associated with situations where large populations of living *Vibrio* species are present on seafoods, either in the raw state where fish and shellfish are eaten uncooked or in decontaminated cooked products which are stored at temperatures that permit growth. However, infective population levels of *Vibrio* can occur on freshly harvested fishes, particularly where water quality is poor and temperatures are high. Contaminated shellfish may be a source of hepatitis. Smoking is the preferred method of fish preservation in most rural areas and riverine fishing communities especially in the Delta State of Nigeria.

2. Materials and Methods

Three common fish species were used in this study; they are *Merluccius merluccius* (Merluza), *Sardina caerulea* (Scombia) and *Lulilus comutus* (Sese). These species were chosen for this work because they are commonly sold in Benin City. Samples were purchased from open markets and stores in Uselu market, New-Benin and Oba market in Benin City and placed in labelled clean containers and transported to the laboratory for analysis. Ten grams (10g) of fish sample was macerated in a mortar and then dissolved in a test-tube containing 90ml of sterilized distilled water to obtain a solution. 0.1ml aliquots of 10^{-1}, 10^{-2} and 10^{-3} dilution were aseptically removed with a sterile pipette and transferred into labelled sterile Petri dishes and then about 18 – 20ml melted Nutrient agar were added by pour plate method. After rotating gently, the plates were incubated at 37°C for 24hrs. Enumeration of different plate was carried out and the total mean count was determined and recorded in cfu/g. Selected colonies were transferred from mixed culture onto fresh nutrient plates using inoculating loop and then incubated at 37°C for 24hrs. 0.1ml aliquot of 10^{-1}, 10^{-2} and 10^{-3} dilution were aseptically removed with a sterile pipette and transferred to labelled Petri dishes, and then about 18 – 20ml melted MacConkey agar was added by pour plate method. After rotating gently, the plates were incubated at 37°C for 24hrs. Enumeration of different plates were carried out and then the total mean count was determined and recorded in cfu/g. Selected colonies were transferred from the mixed culture onto fresh plates and incubated at 37°C for 24hrs. Aliquot of 0.1ml of the 10^{-1}, 10^{-2} and 10^{-3} dilution were pour plated in Potato dextrose agar which was supplemented with penicillin to inhibit bacterial growth. The plates were incubated at 28±2°C and observed daily for about 3days. All observed colonies were sub-cultured and identified. Each different appearing fungal culture isolate was transferred with a sterile needle to a sterile slide, stained with a drop of lacto phenol cotton blue and then examined macroscopically and microscopically to aid identification.

Bacteria isolates were identified using various biochemical tests such as citrate utilization test, urease test, catalase test, indole test, oxidase test and coagulase test. Bacteria counts for both smoked and fresh fish were subjected to (t-test) statistical analysis. The pH of fish was determined using a digital pH meter after blending 10g of homogenised fish with 90 ml of distilled water. The moisture content of sample was determined using the AOAC official methods for analysis [7]. Ten grams (10g) sample was weighed in a pre-weighed ceramic crucible on an analytical balance and dried to a constant weight at 105°C for 24 hours in an oven. The sample was allowed to cool to room temperature in a desiccator and was accurately weighed to determine the dry weight. The loss in weight was attributed to moisture content. Moisture content was calculated as follows:

$$\text{Percentage } (\%) \text{ moisture } = \frac{W_2 - W_3}{W_2 - W_1} \times 100\%$$

W_1= Weight of empty crucible in grams,
W_2= Weight of sample + crucible before drying in grams
W_3= Weight of sample + crucible after drying, in grams.

3. Results and Discussion

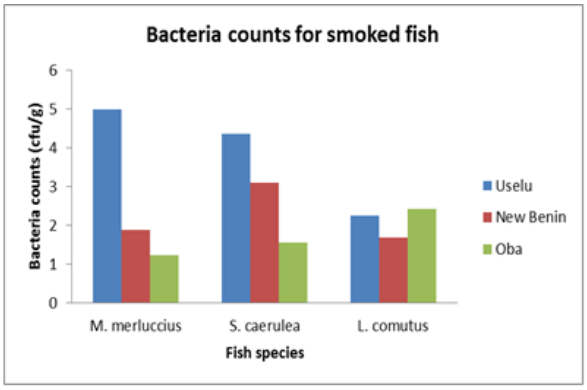

Figure 1. Bacteria counts for smoked fish

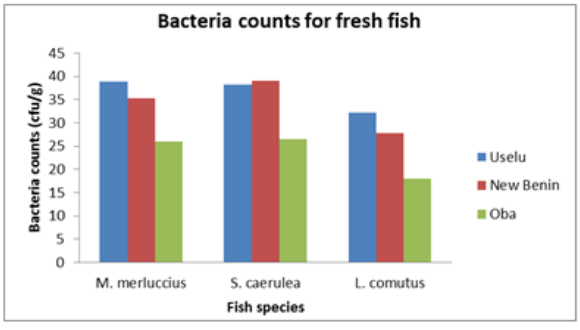

Figure 2. Bacteria counts for frsh fish

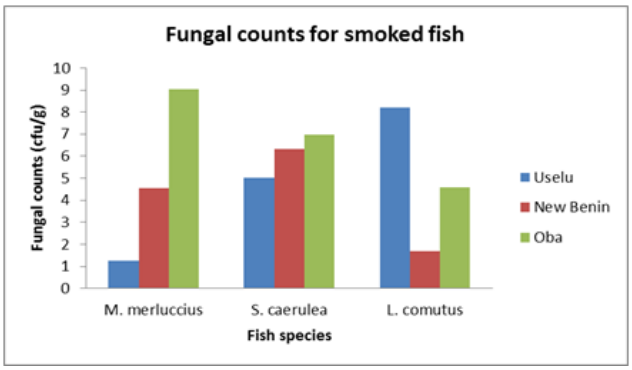

Figure 3. Fungal counts for smoked fish

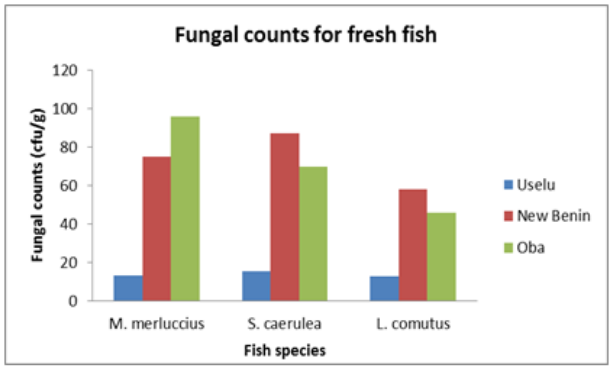

Figure 4. Fungal counts for fresh fish

Table 1. Bacterial and Fungal isolates

Bacteria isolates		Fungal isolates
Fresh fish	Smoked fish	Fresh / Smoked fish
Corynebacterium spp.	*Escherichia coli*	*Penicillium expansum*
Enterobacter spp.	*Proteus* spp.	*Saccharomyces* sp.
Flavobacterium spp.	*Bacillus subtilis*	*Aspergillus niger*
Acinetobacter spp.	*Micrococcus luteus,*	*Fusarium* spp.
Escherichia coli	*Pseudomonas aeruginosa*	*Rhizopus stolonifer*
Proteus spp.	*Serratia* sp.	*Mucor piriformis*
Bacillus subtilis	*Streptococcus pyogenes*	
Micrococcus luteus,	*Staphylococcus aureus*	
Staphylococcus epidermidis		
Serratia sp.		
Pseudomonas aeruginosa		

Table 2. pH value and moisture content of smoked and fresh fish

	Smoked fish	Fresh fish
pH value	5.87±0.06 – 6.63±0.02	6.57±0.003 - 6.83±0.003
Moisture content	12.62±0.14 – 33.91±0.34	52.95±0.64 – 63.30±0.13

This study revealed that the microbial count of the smoked fish samples ranged from 8.344 x 10^5 – 3.108 x 10^6 for bacteria and 1.693 x 10^5 – 9.043 x 10^5 for fungal which is less than that of the fresh fish samples which ranged from 2.774 x 10^6 – 4.416 x 10^6 for bacterial and 5.787 x 10^5 – 1.840 x 10^6 for fungal (Figure 1, Figure 2, Figure 3 and Figure 4). Statistically, t-test showed that there is a difference in the microbial load of smoked and fresh fish. This could be due to sanitary conditions under which the fish samples are handled and kept [8]. Also, this study revealed that moisture content and pH influenced the microbial load of fish samples. The moisture content and pH values of smoked fish ranged from 12.62±0.14 – 33.91±0.34 and 5.87±0.06 – 6.63±0.02 respectively. For the fresh fish samples, moisture content and pH values ranged from 52.95±0.64 – 63.30±0.13 and 6.57±0.003 – 83±0.003 respectively (Table 2). The bacteria isolated from the Uselu and New Benin market had higher compared to that Oba market while fungal isolates from Oba was higher than those of Uselu and New Benin market. Several bacteria and fungi were isolated from the fresh and smoked fish samples (Table 1). The result of this study revealed that *Staphylococcus aureus* and *Bacillus subtilis* were the common pathogenic bacteria found associated with fresh and smoked fish in Benin metropolis. The presence of *Staphylococcus aureus* was attributed to the contamination of the fish samples by man through handling and processing. [9] recorded that *Staphylococcus aureus* occurs as natural microflora of fish and shellfish. In a similar study carried out by [10], *Bacillus subtilis, Staphylococcus aureus, Proteus mirabilis, Klebsiella* sp., *Salmonella typhii* and *Streptococcus* sp. were all found to be associated with smoked fish. It was suspected that these organisms may have contaminated the smoked fish through human handlers, air and soil. The presence of *Enterobacter* sp. could be indicative of feacal contamination. Some *Bacillus* sp. are pathogenic and can cause food poisoning, bacteraemia and endocarditis, their presence in some of the fresh and smoked fish samples may be because they are sold openly in the market and are exposed to the spores of the organism which are dormant in that environment and are highly resistant to the lethal effects of heat drying and ultraviolet radiation [11]. *Staphylococcus aureus* is capable of producing enterotoxin and is noted to survive for extended periods in hostile environments [11,12]. It could cause gastroenteritis in the individuals if the fish is consumed raw. [8] also isolated and identified *Staphylococcus aureus, Bacillus* sp., *Salmonella* sp. and *Streptococcus* sp. from the skin of *Clarias gariepinus which* also supports the outcome of this study. *Bacillus* sp., *E. coli, Salmonella* sp., *Streptococcus* sp. and *Staphylococcus aureus* have been implicated in fish-borne diseases of humans [13]. In a study carried out by [14] on bacterial infection of mudfish *Clarias gariepinus,* the predominant bacteria isolated were *Staphylococcus aureus, Escherichia coli* and *Pseudomonas*

fluorescens. Salmonella sp. Which were recovered from gills, intestine and whole body of catfish *Clarias gariepinus* and sea food in Malaysia [15,16,17]. Although *Salmonella* sp. was not isolated in this work, its presence in fish constitutes a food safety problem because catfish could be a potential agent of transfer of these species to unsuspecting customers. The occurrence of *Aspergillus* sp, *Rhizopus* sp., *Fusarium* sp. and *Penicillium* sp. in the smoked fish species could be due to the fact that during storage, the fish sample reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing, handling and display on the market stalls [18].

Microbial contamination of smoked and fresh fish has been found to be due to several factors such as poor smoking of fish products (inappropriate temperature control or application), poor personal hygiene of processors/seller, poor hygiene/sanitary practices relating to smoked fish products, smoke/workhouse, packaging and storage as well as the use of inadequate and inefficient traditional processing facilities. Poor environmental sanitation and high human handling by buyer and seller are also implicated. There was no significant difference in the microbial contaminants among the fish species. However, statistically, the microbial counts of the fresh fish samples were comparably higher than those of the smoked fish samples which could be probably due to the smoking effect. This means that properly smoked and well preserved fish is safe for eating and free from microbial contamination compared to that of the fresh fish. Also, this study revealed that moisture content and pH greatly affects the microbial load and microorganisms isolated. Therefore, this study advocates the need for the adoption of good processing practices and preserving of smoked fish product, proper hygiene and storage of both smoked and fresh fish, high safety standards to maintain the market worthiness of the final products.

Conflict of Interests

No potential conflict of interests was disclosed.

References

[1] Feldhusen, F. *The role of sea food bacterial food-borne diseases*. Microbes and Infections, 2000, 2: 1651-1660.

[2] Ayuba, V.O. and Omeji, N. O. *Effect of insect infestation on the shelf life of smoked and dried fish*. Proceedings of the 21st Annual Conference of the Fisheries Society of Nigeria (FISON), Calabar, 13-17th November, 2006, P. 357-359.

[3] Okonta, A. A. and Ekelemu, J. K. *A prelimnary study of micro-organisms associated with fish spoilage in Asaba, Southern Nigeria.* Proceedings of the 20th Annual Conference of The Fisheries Society of Nigeria (FISON), Port Harcourt, 14th-18th November, 2005, p 557-560.

[4] Ali, A., Karunasagar, I. and Karunasagar, I. *Bacteriological changes during iced storage of the tropical freshwater carp, Labeo rohita*. Fish. Res., 1992, 13: 189-197.

[5] Thrower, S. J. *Handling practices on onshore fishing vessels: Effects on the quality of finfish products.* Food Research, 2000, 47:50-55

[6] Shewan, J. M. *The Bacteriology of fresh and spoiling Fish and the Biochemical changes induced by Bacterial action.* Tropical Products Institute, London, 1977, 66 pp.

[7] AOAC. *Official methods for analysis* (volume 1), 1990, 9 CFR 318.19(b).

[8] Tiamiyu, A. M., Emikpe, B. O. and Adedeji, O. B. *Isolation and Identification of aerobic bacteria flora of the skin and stomach of wild and cultured Clarias gariepinus and Oreochromis niloticus from Ibadan, Southwest, Nigeria*. Journal of Applied Sciences Research, 2011, 7(7):1047-1051.

[9] Claucas, I. J. and Ward, A. R. *Post-harvest Fisheries Development: A Guide to Handling, Preservation, Processing and Quality*. Chartan Maritime, Kent. ME4 TB, United Kingdom,1996, 276 pp

[10] Moshood, A. Y. and TengkuHaziyamin, A. A. *Isolation and Identification of Bcateria in Retailed Smoked Fish within Bauchi Metropolis.* Journal of Pharmacology and Biological Sciences, 2012, 3 (1): 1-5

[11] Doyle, M. P., Beuchat, K. R. and Mont-Vile, T. J. *Food Microbiology*. ASM Press Washington D.C, USA, 1997, 768 pp.

[12] Ehiri, J. E., Azubike, M. C., Ubbaonu, C. N., Anyanwu, E. C., Ibe, K. M. and Ogbonna, M. O. *Bulletin of World Health Organization*, 2001, 79(5):423-433.

[13] Babu, P. S. *Ichyozoonoses*. Fish farmer International, 2000, 14: 14-17.

[14] Ipki, G. U. and Offem, B. O. *Bacterial infection of cultural fishes in the fish farm of the Cross River University of Technology*. Egypt Journal of Microbiology, 2008, 21: 57-63.

[15] Bremer, P. J., Fletcher, G. C. and Osborne, C. New Zealand Institute for Crop and Food Research Ltd., New Zealand, 2003, 12 pp.

[16] Kumar, R., Surendran, P. K. and Tampuran, N. *Distribution and genotypic characteristics of Salmonella serovars isolated from tropical sea food of Cochin.* India Journal of Applied Microbiology, 2009, 106: 515-524.

[17] Ponce, E., Khan, A. A., Cheng, C. M., Sumange-West, C. and Cerniglia, C. E. *Prevalence and Characterisation of Salmonella enterica serovar Welevreden from imported sea food*. Food Microbiology, 2008, 1: 29-35.

[18] Christianah, I., Ayolabi, O. and Fagade, O. E. (2010). *Mycological Evaluation of Smoked Fish from the Retail Outlets in Ago-Iwoye, Ogun State, Nigeria*. Journal of Life and Physical Science, 2010, 3(2): 65-66.