Sensitivity of Bacteria Isolated from Smoked Cayenne Pepper (*Capsicum minimum*) Treated *Clarias gariepinus*

Adeosun O*, Olateju GB* and Akinyemi AA

1Department of Fisheries Technology, Oyo State College of Agriculture and Technology, Igboora, Nigeria
2Department of Aquaculture and Fisheries, Federal University of Agriculture, Abeokuta, Nigeria

**Abstract**

Microbiological examination of fish aims at evaluating hygienic quality of fish, including temperature abuse, and the possible presence of pathogenic microorganisms in the fish. Microbial activity is responsible for spoilage of most fresh and of several light preserved sea foods. This paper evaluates the sensitivity of bacteria isolated from smoked *Clarias gariepinus* to different concentrations of ground Cayenne pepper and commercial antibiotics. The bacteria isolated from the samples are *Bacillus subtilis*, *Staphylococcus saprophyticus*, *Escherichia coli* and *Micrococcus* species. *Bacillus subtilis* was the most abundant found in the fish samples throughout the four weeks of storage while *Micrococcus* species was the least occurring bacteria all through the four weeks of storage. Sensitivity results revealed that all bacteria isolates were resistant to all levels of cured smoked fish samples with pepper except *Escherichia coli* which was intermediate to 1% pepper. It was noticed that as the level of the pepper increases, the bacteria isolates showed resistance which infers that the less the pepper, the more effective it is to inhibit bacteria isolates. Results of effect of commercial antibiotics on the bacteria isolated from the smoked cured fish samples showed that all bacteria isolates were significantly susceptible to Ofloxacin and Pefloxacin while Amoxicillin was the least effective in all the commercial synthetic antibiotics used. The findings of this study revealed a lower microbial count. The bacteria load obtained from the smoked fish fell within range. The microorganisms isolated and identified from the fish samples can be said to be normal flora of the fish.

**Keywords**: Pefloxacin; *Bacillus subtilis*; Sulphides; Metabolism

**Introduction**

Fish and fisheries products are among the most perishable commodities worldwide mainly due to microbial spoilage in fish [1]. Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines, such as organic acids, sulphides, alcohols and ketones with unpleasant and unacceptable off flavours [2,3]. Composition of micro-floral on newly catch fish depends on the microbial content of the water in which the fish live. Fish micro-flora includes bacteria in fish such as *Pseudomonas*, *Alcaligenes*, *Vibrio serrita* and *Micrococcus* [3].

**Materials and Method**

12 kg table size *Clarias gariepinus* samples were selected for the experiment. The fish samples were killed, gutted, washed and allowed to drain. Subsequently, they were divided into two batches A and B. Fish samples in batch A were treated with salt and this serves as control. Fish in batch B were divided into six (6) groups of five (5) sample each and soaked in 1%, 2%, 3%, 4%, and 5% cayenne pepper solution for 10 minutes respectively. The fish were drained and smoked using CORAF/WECARD smoking kiln for 10 hours at a temperature ranging from 75-85°C and after smoking fish were allowed to cool to room temperature and stored in different boxes. This was done to mimic commercial practices. Samples were drawn weekly from the control and treated sample for four weeks. Smoked fish samples were analysed for Total Bacterial count on Nutrient Agar, Isolation of microorganisms from the stock culture and Sensitivity of isolated bacteria to pepper and commercial antibiotics was determined using the method of Clinical and Laboratory Standards Institute (2012).

**Results**

The microbial load of bacteria isolated from the smoked cured fish samples at different levels (1-5%) during the storage periods are as presented in Table 1. The total bacteria count ranged from 0.0 to 0.8 × 10^6 CFU/g. The total bacterial count obtained in this study was lower than the recommended values by the International Commission on Microbiological Specifications for foods [4]. Table 2 revealed the occurrence of bacteria isolates in the fish samples with the control smoked cured samples harbouring all the bacteria isolates: *Bacillus subtilis*, *Staphylococcus saprophyticus*, *Escherichia coli* and *Micrococcus* species. *Bacillus subtilis* was the most abundant found in the fish samples throughout the four weeks of shelf life while *Micrococcus* species was the least occurring bacteria all through the four weeks of storage. Table 3 revealed that all bacteria isolates were resistant to all levels of cured smoked fish samples with pepper except *Escherichia coli*.

**Table 1**: Average bacteria count of fish samples from week one to week four of shelf life.

**Table 2**: Sensitivity of bacteria isolated from smoked Cayenne Pepper (*Capsicum minimum*) Treated *Clarias gariepinus*.

**Table 3**: Sensitivity of *Bacillus subtilis* isolated from smoked Cayenne Pepper (*Capsicum minimum*) Treated *Clarias gariepinus*.

*Corresponding author: Adeosun O, Department of Fisheries Technology, Oyo State College of Agriculture and Technology, Igboora, Nigeria, Tel: +234 803 804 1713; E-mail: moriyike2006@gmail.com

Received May 20, 2018; Accepted June 02, 2018; Published June 05, 2018

Citation: Adeosun O, Olateju GB, Akinyemi AA (2018) Sensitivity of Bacteria Isolated from Smoked Cayenne Pepper (*Capsicum minimum*) Treated *Clarias gariepinus*. J Fisheries Livest Prod 6: 273. doi: 10.4172/2332-2608.1000273

Copyright: © 2018 Adeosun O, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
coli which was intermediate to 1% pepper. It was noticed that as the level of the pepper increases, the bacteria isolates showed resistance which infers that the less the pepper, the more effective it is to inhibit bacteria isolates. Table 4 showed the effect of commercial antibiotics on the bacteria isolated from the smoked cured fish samples. All bacteria isolates were significantly susceptible to Ofloxacin and Pefloxacin while Amoxycillin was the least effective in all the commercial synthetic antibiotics used.

Discussion

This study showed that pathogenic bacteria are present in the smoked cured Clarias gariepinus. According to International Commission on Microbiological Specification for Food [4], the maximum recommended bacteria count for good quality product is $5.0 \times 10^6$ $(5.7 \log \text{cfu/g})$. The findings of this study revealed a lower microbial count, compared to that obtained for the skin, intestine and gills of fish samples in an investigation [5,6] - the bacterial load ranged from $10^5$ to $10^7$ cfu/g. Higher microbial level was reported by Al-Harbi and Uddin (2008) ranging from $3.5 \times 10^5$ to $1.6 \times 10^6$ cfu/g in gill filaments and $8.7 \times 10^5$ to $5.4 \times 10^6$ cfu/g in intestines of common carp, Cyprinus carpio cultured in ponds in Saudi Arabia. Similar trend of bacterial growth has been reported by several workers. In a study of microbial load of fresh fish samples in Benin City, a range of 2.8 × 10⁶ – 4.4 × 10⁶ for bacterial count was recorded [7].

The bacteria load obtained from the smoked fish fell within range. The microorganisms isolated and identified from the fish samples can be said to be normal flora of the fish e.g. Bacillus sp. [8]. The normal microbial flora of the fish are not initially harmful, as they even help in preventing the invasion of the fish flesh by other microorganisms but they become pathogenic when there is an enabling environment that promotes their growth. Bacteria present in the fish samples include, Bacillus subtilis, Staphylococcus saprophyticus, Eschericia coli and Micrococcus species. The occurrence of Staphylococcus saprophyticus and Eschericia coli in the smoked fish samples had been reported by Martin [9] when he stated that these organisms were the commonest micro-organisms associated with smoked fish. The presence of

| Organisms                        | 1% | 2% | 3% | 4% | 5% | CPX |
|----------------------------------|----|----|----|----|----|-----|
| Staphylococcus saprophyticus     | 0  | 0  | 0  | 0  | 0  | 36  |
| Bacillus subtilis                | 0  | 0  | 0  | 0  | 0  | 37  |
| Escherichia coli                 | 13 | 11 | 4  | 10 | 0  | 34  |
| Micrococcus species              | 12 | 2  | 5  | 4  | 0  | 36  |

Table 2: Occurrence of bacteria in smoked cured fish samples.

Table 3: Sensitivity of bacteria isolates to different treatments of pepper.

| Treatment      | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 |
|----------------|--------|--------|--------|--------|--------|
| Control        | Escherichia coli | Escherichia coli | Bacillus subtilis, Staphylococcus saprophyticus | Bacillus subtilis | Bacillus subtilis, Staphylococcus saprophyticus |
| 1% Bacillus subtilis | Bacillus subtilis | Bacillus subtilis, Staphylococcus saprophyticus | Bacillus subtilis | Nil | Nil |
| 2% Staphylococcus saprophyticus | Staphylococcus saprophyticus | Bacillus subtilis | Nil | Nil |
| 3% Bacillus subtilis, Micrococcus species | Bacillus subtilis, Micrococcus species | Bacillus subtilis | Nil | Nil |
| 4% Escherichia coli | Escherichia coli | Nil | Nil |
| 5% ND | ND | Micrococcus species | Nil | Staphylococcus saprophyticus |

Ciprofloxacin (10UGmL-1) (Positive control)

Resistance: 0-12; Intermediate: 13-18; Susceptible: 19-Above (CLSI, 2012).

Table 4: Sensitivity of bacteria isolates to commercial antibiotics.

Staphylococcus saprophyticus in fish samples according to Okonko et al. [10] might have been through contamination by handling.

The bacteria group Staphylococcus according to Herman et al. [11] reported that it was one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane without resulting to a disease condition. This bacteria class may also cause superficial and systemic infections such as boils, impetigo and folliculitis while more serious and more common infections could be pneumonia, bacteremia and other infections of the bones and wounds. Also, Escherichia coli usually cause diarrhea and kidney damage as well as uncomplicated community acquired urinary tract infections. The presence of the organisms could be as a result of handling during smoking and also cross contamination during storage, after smoking and handling during sales of smoked fish.

According to Thampuran et al. [2], E. coli is commonly associated with sea food contamination in the tropics, where it is encountered in high numbers. Thampuran et al. [2] isolated E. coli in finfish samples acquired at the retail market, and although typical E. coli or labile toxin-producing E. coli was not detected, the isolation of strains with the ability to produce hemolysis in human blood was a fact worth
mentioning. Marin et al. [12] detected E. coli when researching the bacteriological quality of Cynoscion quambipinnis and Lutj anus gutattus fish samples marketed in Costa Rica. Koo et al. [13] reported having isolated pathogenic strain from rockfish sold in South Korea, and alerting to the presence of E. coli pathogen in seafood.

All bacteria isolates were significantly susceptible to Ofloxacin and Pefloxacin. It was revealed that the microorganisms are resistant to different treatments of pepper. The use of antimicrobial agents in aquaculture and then possibility of antibiotics resistance among bacteria flora from fish have been identified [14,15].

References

1. Oluborode GB, Omorinkoba WS, Bwala RL (2010) Development and construction of an electrical furnace and control system for fish drying. Afra J Eng Res Dev (Devon Science Publication) 3: 123-128.
2. Thampuran N, Surendraraj A, Surendran PK (2005) Prevalence and characterization of typical and atypical Escherichia coli from fish sold at retail in Cochin, India. J food protection 68: 2208-2211.
3. Gram L, Huss HH (2011) Fresh and processed fish and shell fish. In The Microbiological Safety and Quality of Foods, Lund BM, AC Baird-Packer and GW: Gould (Eds) Chapman and Hall, London: 472-506.
4. International commission on Microbiological specification for foods ICMSF (1986) Microorganisms in foods 2, Sampling for Microbiological Analysis. Principles and Specific Applications, second (ed.), Blackwell Science, Oxford.
5. Sugita H, Tsunohara M, Ohkushi T, Deguchi Y (1998) The establishment of an intestinal microflora in developing goldfish (Carassius auratus) of culture ponds. Microbiology Ecol 15: 333-344.
6. Zymslowa L, Lewadowska D, Guzir J (2010) Arch Pol Fish 8: 259-269.
7. Udochukwu U, Udochukwu J, Inetianbor SO, Akaba FO, Omorotionwman J (2016) Comparative Assessment of the Microbiological Quality of Smoked and Fresh Fish Sold in Benin City and Its Public Health Impact on Consumers. Am J Microbiol Res 4: 37-40.
8. Ola JB, Oladipo A (2004) Storage Life of Croaker (Pseudotholitus senegalensis) in Ice and Ambient Temperature, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria. Afr J Biomed Res 7: 13-17.
9. Martins AM (1994) Fisheries Processing: Biochemical Applications. Published by Chapman and Hall, London: 1-88.
10. Okonko TA, Ogumusu AA, Ogunjobi AO, Adedeji OD, Adejoye ET, et al. (2008) Microbial Studies on Frozen Shrimps Processed in Ibadan and Lagos, Nigeria. Scientific Research 3: 543-545.
11. Herman HH, Lin W, Petrecca PJ, Simmons MR, Houghton J (2011) Centrifugal Bioreactors and their application in remediation. Remediation Journal 11: 15-33.
12. Marin C, Fonseca C, Arias S, Villegas I, Garcia A, et al. (2009) Bacteriological load of the fishes Cynoscion quambipinnis and Lutj anus gutattus in the marketing chain, Costa Rica Rev Biol Trop 57: 45-52.
13. Koo HJ, Kwak HS, Yoon SH, Woo GJ (2012) Phylogenetic group distribution and prevalence of virulence genes in Escherichia coli isolates from food samples in South Korea. World J Microbiol Biotechnol 28: 1813-1816.
14. Midvedt T, Lingaas E (1992) Putative public health risk of antibiotics resistance development in aquatic bacteria. In: Michael C, Alderman DJ (ed) “Chemotherapy in aquaculture; from theory to reality” Office International de Epizooties: 302-314.
15. Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI document M100-S25.