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HEAVY METAL LEVELS AND MICROFLORA OF CRAYFISH AND STOCKFISH DISPLAYED FOR SALES

[1]OMORODION NNENNA J. P, [2]OGWU, PECULIAR P

University of Port Harcourt, Department of Microbiology PMB5323 River state Nigeria.

Corresponding author E-mail: nnenna.omorodion@uniport.edu.ng, _nnennaomorodion@gmail.com

Abstract: **Stock fish and crayfish in a typical Nigerian society is a major requirement in meals as they are good sources of protein and other nutrients. This study investigated the microflora and heavy metal levels of stockfish and crayfish sold in the market. 50 samples of stock fish and crayfish were analyzed using standard microbiological techniques. Stockfish parts had total heterotrophic bacterial counts (THBC) ranging from $3.5x10^4$ to $8.4x10^5$ Cfu/g while crayfish samples had THBC ranging from $3.2x10^4$ to$4.5x10^6$ Cfu/g. Only one stock fish sample showed sparce growth of *Aspergillus* sp. Bacterial genera obtained from the study and their frequencies included *Bacillus* sp. (21.6 %), *Staphylococcus* sp. (19.6 %), *Micrococcus* sp. (27.5 %), *Proteus* sp. (5.9 %), Klebsiella sp., (15.6 %), *Pseudomonas* sp. (3.9 %) and *Salmonella* sp. (5.9 %). Heavy metal compositions of samples were all within acceptable values. Heavy level showed that stockfish samples had more heavy metal constituent compared to the crayfish in our study except for Lead which was higher in crayfish compared to stockfish This study indicates that the samples were contaminated with bacteria which have been reported to be pathogenic to humans hence adequate hygiene must be observed during the collection, processing, transportation and sale of displayed stock fish and crayfish in market places is recommended.**

Keywords: **Crayfish, Stock Fish, Heavy Metal Level, Microbiological Quality.**

1. INTRODUCTION

Fish are aquatic animals that habit, survive and carry out their daily activities in water. There are several species of fishes distributed all over the world, they belong to the kingdom Animalia, phylum chordata and subphylum vertebrata. All the species of fish found in the world are classified into Agnatha (jawless fish), Chrondrichyes (cartilaginous fish), Osteirchthyes (bony fish), Ray finned and Lobe finned fish. Fish is a good source of protein, lipid, fat, essential fatty acids, minerals and vitamins (Paul *et al*., 2018).

Stock fish is made from different species of white fish but cod is the most frequently used fish, cod fish belong to a group of fish called Osteirchthyes meaning bony fish. There are several species of cod, they are named according to their habitats and other characters (such as colour, structure and swimming pattern) they include; Gradus morhua (Atlanta cod), Molva molva (lingcod), Green cod, Pacific cod, Pollachius vireus (saith), Tusk (Brosme brosme), Pollachius pollachius, Haddock (Melaogrammus aeglefinus), Vicenza cod, Bacalhau cod, Norwegian cod, Murray cod and Alaska Pollock (Akin *et al.*,2015).

The cod used in stock fish production are usually harvested in fresh water so they are usually salted two weeks before drying. According to Abiodum *and Blessing* (2016) stock fish is one of the foreign fish which are distributed to many countries mostly in Nigeria, this is because the fish is not found in any part of the country, the fish is locally known as Okporoko among the Igbos, Bazabaza among the Benins and kpanla among the western part of the country. Fish generally are important source of protein and vitamins needed by human and animals to maintain tissues and cells, and it's mostly consumed more than meat claimed (Akinwumi and Kehinde, 2015).

Crayfish are eaten all over the world and only a portion of the body is edible, it's a special ingredient for many foods due to its taste and nutrient value. Crayfish is packed with high quality protein, amino acids, vitamins and minerals just as human enjoy crayfish due to its great taste and nutrient values, microorganisms also colonize crayfish because they possess the adequate nutrients needed for their growth and survival.

In Nigeria there are three (3) species of crayfish found in Cross River state, Bayelsa, River and Delta. They include: White crayfish (from the family of procambarus acutus),Prawn crayfish (red big crayfish locally known as Oporo) and from the family of procambarus clarkii.Shrimp (small red crayfish locally known as native crayfish) from the family of procambarus clarkii and it's known as juveniles because of their size. Crayfish and Stockfish are contained of essential fatty foods and other class of Aminos essential to human but needed to balance body's functions, fishes also contained pack of minerals and trace metals along large portion of protein and fat. (Emad *et al*., 2012). Sometimes these fishes may contain other chemicals not mentioned above, this may happen following the release of harmful chemicals from industries and once these chemical contaminants get into aquatic animals, they find their way into the food chain thereby compromising food safety. This study was performed to investigate the microflora and heavy metals level in stock fish and cray fish sold in the market.

2. MATERIALS AND METHOD

Study Area/ sample Collection

A total of 50 samples (30 stock fish and 20 crayfish) were sourced from three markets within Obio-Akpor Local Government Area, of Rivers State and was transported into a sterilized bags and containers to the Microbiology laboratory for analysis.

Serial Dilution of Samples

25g of each sample (Stockfish and Crayfish) were added into 225 ml of peptone water, swirled and allowed to stay for about 3hours on a shaker, after which a ten-fold serial dilution was done by pipetting 1 ml from the stock solution into the next test tube (10^{-2}), the process was done repeatedly up to (10^{-5}). The swab samples were soaked in 1ml of peptone water and subsequently the 1ml was transferred into 9mls diluents to dilute the sample. 10mls of water samples were introduced into 90mls of peptone water to enrich and then 1ml was transferred for serial dilution.

Isolation of Microorganisms

From the prepared diluents, 0.1 ml of each last two prepared dilutions were transferred into sterile Petri plates containing the different media used and was spread gently using sterile glass rod. The plates were incubated at 37^{o} C for 18-24 hours (h) for the bacteriological media which include Plate count agar, MacConkey agar, Mannitol salt agar used for bacteria growth and Potato dextrose agar for fungi isolation.

The microbial count for each sample was obtained from the previously incubated Petri plates and was expressed as a colony forming unit (cfu/g and cfu/ml). Single colonies of bacteria growth on plat count agar were randomly selected from different media plates based on their morphology and were sub cultured and incubated at 37^{o}C for 24 hours (h) to obtain pure colonies. Isolates were identified based on their morphological and cultural characteristics on growth media. Identification materials, reagents and protocols according to (Cheesebrough, 2000) were used to identify discrete colonies from the bacteriological media of sub-cultured isolates.

The cultural characteristics of each fungi isolates were identified according to their colour, shape and the cell morphology was done based on mycelia, hyphae, septate, spore formation using lactophenol blue. A piece of the mycelium from the Petri plates was mounted on a clean grease free slide using a sterile wire loop and covered with a cover slip, after which a drop of lactophenol cotton blue was added and examined with the microscope.

Determination of heavy metal level was done as described by Agbabiaka and Ibemenuga (2021)

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3. RESULTS

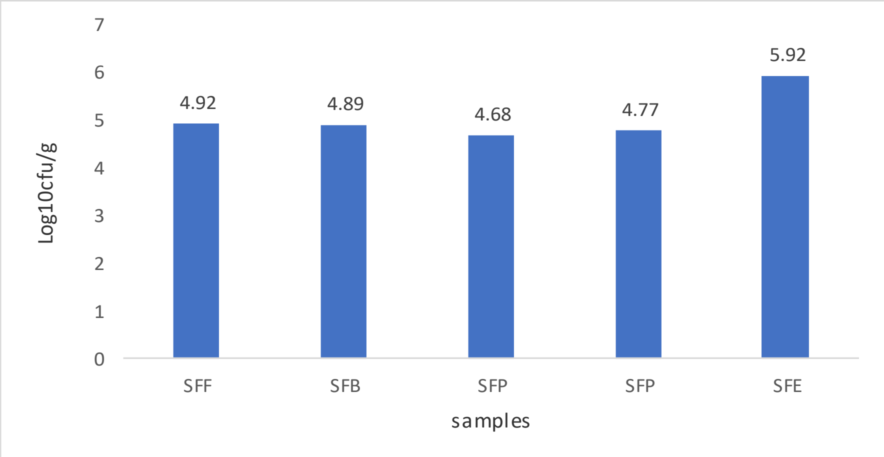

Fig .1: MeanTotal Heterotrophic Bacteria Count of Stockfish Parts

SFF= stock fish flesh, **SFB**= Stock fish bone, **SFP**= Stock fish pieces, **SFE**= Stockfish ear, **SFH**= Stock fish head.

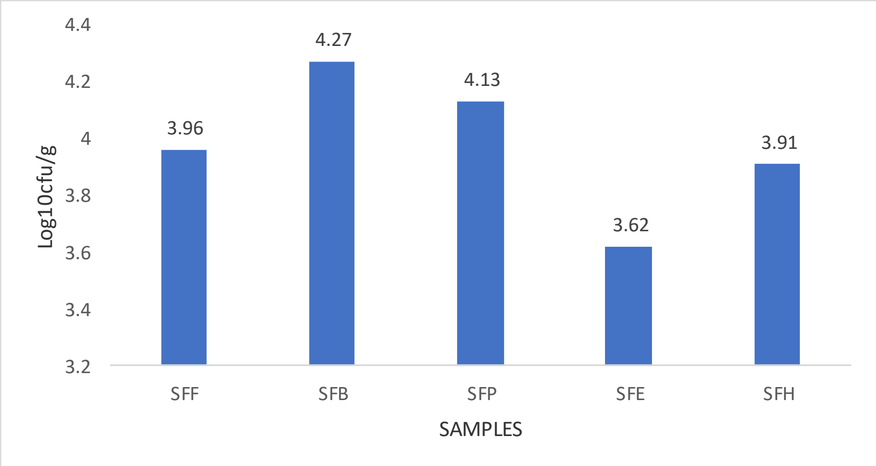

Fig 2: Mean Total Staphylococcus Count of Stockfish Parts

SFF= stock fish flesh, **SFB**= Stock fish bone, **SFP**= Stock fish pieces, **SFE**= Stockfish ear, **SFH**= Stock fish head.

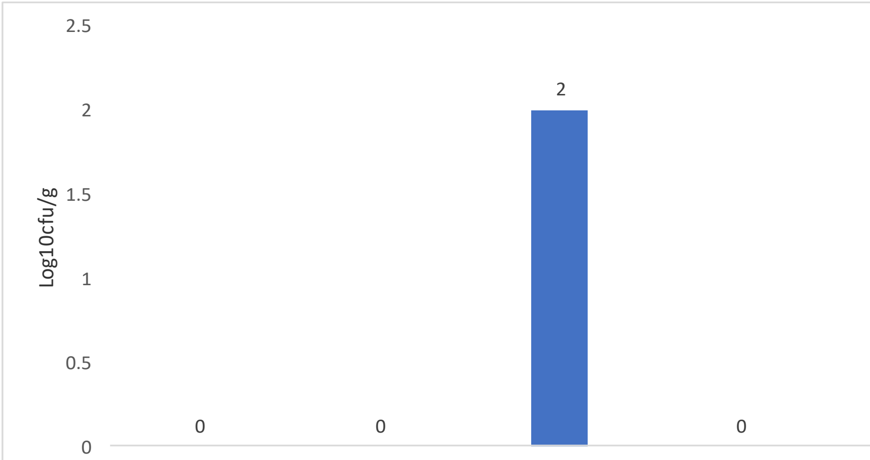

Fig .3: MeanTotal Fungal Count of Stockfish Parts

SFF= stock fish flesh, **SFB**= Stock fish bone, **SFP**= Stock fish pieces, **SFE**= Stockfish ear, **SFH**= Stock fish head.

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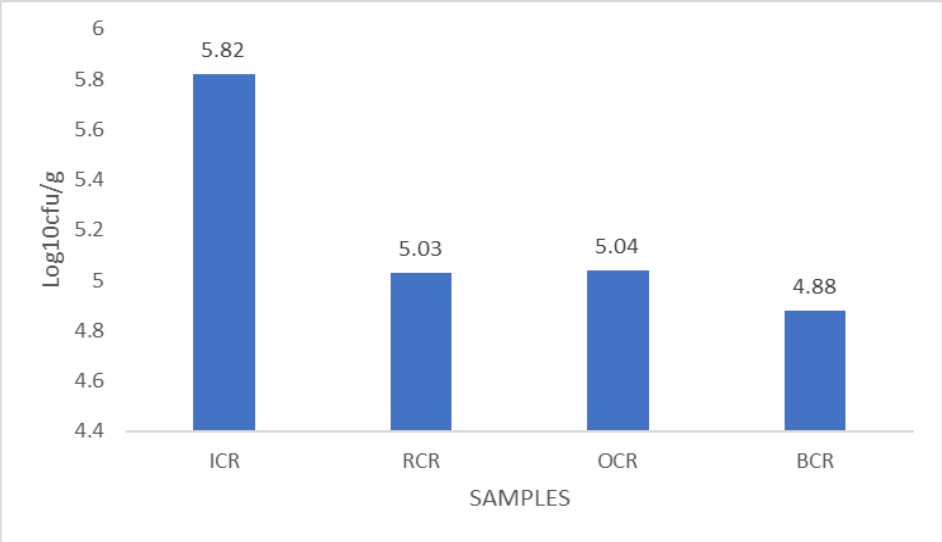

Fig 4: Mean Total Heterotrophic Bacteria Count of Crayfish Samples

ICR= Ijaw crayfish, **RCR**=Rivers, Crayfish **OCR**= Oron Crayfish **BCR**= Big Crayfish(Prawn)

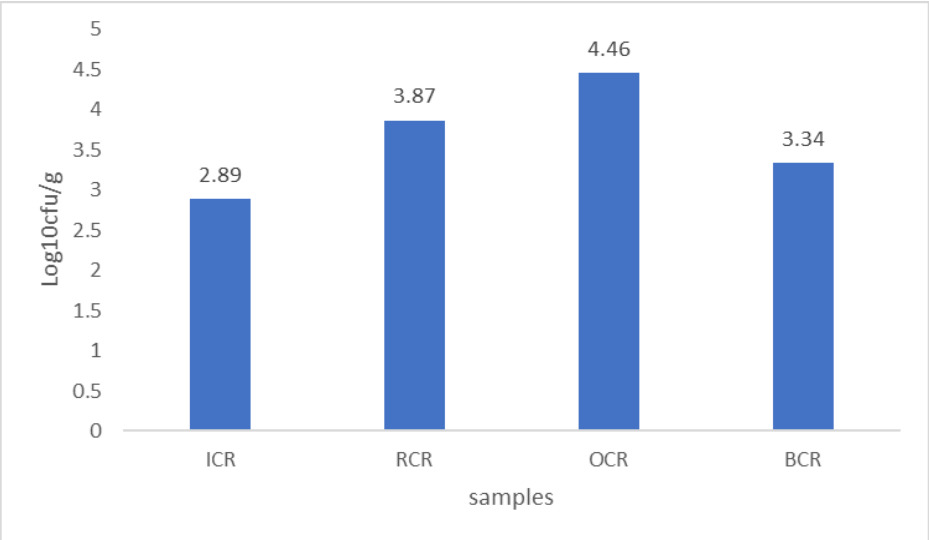

Fig 5: Mean Staphylococcus Count of Crayfish Samples

ICR= Ijaw crayfish, **RCR**=Rivers, Crayfish **OCR**= Oron Crayfish **BCR**= Big Crayfish (Prawn)

Table .1: Frequency of Occurrence of Bacteria Isolated from Stockfish and Crayfish.

Isolates	Crayfish N(%)	Stockfish N(%)	Total N (%)
Bacillus sp.	5 (25.0)	6 (19.4)	11 (21.6)
Staphylococcus sp.	4 (20.0)	6 (19.4)	10 (19.6)
Micrococcus sp.	5 (25.0)	9 (29.0)	14 (27.5)
Proteus sp.	0 (0.0)	3 (9.8)	3 (5.9)
Klebsiella sp.	3 (15.0)	5 (16.1)	8 (15.6)
Pseudomonas sp.	0 (0.0)	2 (6.2)	2 (3.9)
Salmonella sp.	3 (15.0)	0 (0.0)	3 (5.9)
Total	**20 (100)**	**31 (100)**	**51 (100)**

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Table .2: Mean Heavy Metal Composition of Stockfish and Crayfish Samples Studied

Sample/Heavy Metal (ppm)	Crayfish	Stockfish
Lead (Pb)	3.76248	1.29680
Cadmium (Cd)	0.14730	0.31425
Manganese (Mn)	1.58114	3.00796
Zinc (Zn)	15.06743	19.46520
Iron (Fe)	18.06743	31.05731

4. DISCUSSION

Stockfish and crayfish are important component of a typical dish, irrespective of age, economic status, religion and educational background in Nigeria (Adesoji, 2019). The affordability of stockfish and crayfish in addition to their nutritional provisions such as animal proteins, poly-saturated fatty acids and micronutrients makes them one of the major dietary components and of high commercial considerations (Mohanty, 2019). Fishes both in their natural habitat or when reared are constantly exposed to numerous microbes (Olayiwola, 2015). These microbes, as parasites create some damages in fish farms and the diseases caused by microbes are accountable for heavy loss (Kousar, 2019). They ultimately could cause fish -borne diseases if not properly processed, stored and sold within the nearest possible time.

This study investigated the micro-flora and heavy metal composition levels of crayfish and stockfish sold in the market. The total viable count obtained from stockfish studied ranged from 4.2 $x10^4$ to 8.4 $x10^5$ cfu/g while the counts obtained for crayfish ranged from crayfish was between $3.2x10^4$ and $4.5x10^6$ cfu/g. There were no significant differences between the counts obtained in the individual parts of the stockfish studied as well as the different types of crayfish studied. These could be attributed to the fact that only microorganisms contained in air that can easily drop on foodstuffs being exposed in the market places and those on the skin that could be on these products due to touch by persons inspect before purchase.

These results showed that the crayfish had more heterotrophic bacterial growth compared to stockfish and could be as a result of preservatives added to stock fish as well as the their lower moisture content that does not permit much microbial proliferation. Our result obtained in crayfish is comparable to the $2.2x10^4$ to 2.5x 10^4 cfu/g reported by Ugwu, (2019) on dry crayfish at Enugu, Nigeria. The total viable counts on crayfish could also be compared to the works done by Kasozi *et al.*, (2016) which shows microbial load of 2.4x 104 cfu/ml to 7.2 x 104 cfu/ml and Israel *et al.*, (2016) who reported the microbial load of crayfish that ranged from 4.2x 10^2 cfu/g to 4.4x 10^2 cfu/g.

Analysis for the presence of coliforms done on all samples revealed that none of the crayfish and stockfish samples had coliform count in them. Dry surface of the samples could be the result as most enteric organism prefers moist environment for growth. The staphylococcus count showed varying results among individual samples studied. All stockfish samples studied showed staphylococcus counts ranging from 3.6 $x10^3$ to 1.90 $x10^4$ cfu/g while that of crayfish saples ranged from $3.15x10^2$ to $4.8x10^4$ cfu/g. Staphylococcus counts obtained in both samples indicated that stockfish samples were more prone to staphylococcus contamination compared to crayfish samples. This may be due to the fact that people who purchase crayfish most often times do not touch them before purchasing except the trader. Little or insufficient researches has been done on staphylococcus count on both samples making it difficult to access other reports by authors who reported counts of staphylococcus.

However, only one fungus with a $1.0x10^2$ cfu/g count was isolated from both the stockfish and crayfish. This was obtained from stockfish bone tied with rubber band. The little or no count obtained for fungus does not agree with works done by Junaid *et al.*, (2010) who reported all their stockfish samples to be contaminated with fungi.

The microbial counts obtained in this study are higher than all available standards for food and fishes except for the zero-coliform recorded from all samples. It is also important to pinpoint the fact that the samples studied are not meant to be eaten raw and hence cooked before consumption. Therefore, there is likelihood of denaturing the microorganisms isolated before consumption.

Seven bacterial genera were isolated all respective samples analyzed in this study and occurred at different frequencies for crayfish and stockfish samples. They include *Bacillus* sp., *Staphylococcus* sp. *Micrococcus* sp. *Proteus* sp. *Klebsiella* sp., *Pseudomonas* sp. and *Cellebiosoccus* sp. *Bacillus* and *Microcococcus* species had the highest occurrence in crayfish samples 5 (25.0%) followed by *Staphylococcus* sp. 4 (20.0%) while *Klebsiella* and *Cellebiosoccus* species both had a frequency of 3 (15.0%). Notwithstanding, the stock fish sample had 6 (19.4 %) *Bacillus* and *Staphylococcus,* 9 (29.0 %) *Micrococcus* sp., 3 (9.8 %) *Proteus* sp., 5 (16.1 %) *Klebsiella* sp. and 2 (6.2 %) *Pseudomonas* sp. the frequency observed in this study is comparable to the 26.6 % *staphylococcus* and 16.67% *Bacillus* reported by Ugwu (2019). The presence of these microorganisms are pointers of potential public health concerns as most of them are pathogenic in nature. There is therefore need for proper cleaning and pasteurization prior to consumption.

Heavy metal analysis done on one representative sample of each of Stockfish and crayfish showed that crayfish had 3.76249 Lead, 0.14730 Cadmium, 18.38169 Iron, 15.06743 Zinc and 1.58114 Manganese content whereas the stockfish sample had 1.29680ppm lead, 0.31425 Cadmium, 31.05731 Iron, 19.46520 Zinc and 3.00796 Manganese. This indicates that the stockfish samples had more heavy metal constituent compared to the crayfish in our study except for Lead which was higher in crayfish compared to stockfish. The Lead, Zjnc and Iron content in this study is relatively higher than those reported by Abiodun and Blessing. (2016). This difference could be attributed to differences in storage time, chemical preservatives as well as source of samples.

Sea food such as stock fish and crayfish were found to be potential vectors in the transmission of opportunistic pathogenic microorganisms. This study confirms the occurrence of different pathogens in samples of crayfish and stockfish and this could be due to improper handling, improper storage and dirty environment in which they are displayed These could pose potential public health risk if not adequately processed hygienically before consumption.

5. RECOMMENDATIONS

1. Proper and hygienic storage of crayfish and stockfish in market places
2. Buyers should not be allowed to touch food stiffs before buying
3. Traders should buy amount of product they can sell off within the shortest time to avoid over storage.

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