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# Application of Hazard Analysis Critical Control Points on Harvested Mangrove Oysters (*Crassostrea gasar*) from Selected Sources in Rivers State

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author GE designed the study, handled the laboratory work and wrote the draft. Author BJOE Modified and approved the design and supervised the research. Author OCE co-supervised the research, read and approved the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The aim of this study was to assess the hazards associated with oyster from two communities in Rivers State and provides an insight at improving the safety of oyster through the application of the hazards analysis critical control points (HACCP) concept in processing freshly harvested mangrove oysters.
**Study Design:** This work is based on completely randomized design with two replications and the average values calculated for mean comparison.
**Place and Duration of Study:** Food and Industrial Microbiology Laboratory, Department of Microbiology, University of Port Harcourt, between October 2018 and March, 2019.
**Methodology:** The proximate composition, pH and bacterial profile of oysters prepared conventionally and that prepared employing critical control points concept determined using standard methods.
**Results:** The proximate composition of oyster meat revealed the following: moisture (83.73%), protein (8.36%), lipid (1.28%), fiber (1.04%), carbohydrate (2.12%) and ash content (3.47%). The average aerobic plate count for Abuloma and Okrika were 5.69 and 6.98 $\log_{10}$CFU/g respectively

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while coliform count was 5.37 and 5.02$\log_{10}$CFU/g respectively. Two bacterial genera (*Staphylococcus* and *Bacillus*) were isolated from oyster processed with HACCP approach at the last critical control point 7, whereas, nine bacterial genera (*Bacillus*, *Streptococcus*, *Vibrio*, *Escherichia*, *Lactobacillus*, *Klebsiella*, *Proteus*, *Staphylococcus* and *Pseudomonas*) were isolated from the conventionally processed oysters.

**Conclusion:** The HACCP concept gave an improved microbiological quality of oyster meat and the safety of oyster and potential economic value.

*Keywords: Oyster; hazards analysis; critical control points.*

## 1. INTRODUCTION

Oyster (*Crassostrea gasar*) is among the most abundantly harvested shell-fish with high value globally. Oyster is an adequate source of protein, minerals (copper, iron, zinc) and vitamins (thiamin, riboflavin, ascorbic acid) [1,2]. It is a source of animal protein for the communities situated on banks of rivers and the public in general, as they are bountiful in the brackish water [2,3,4,5].

In Nigeria, especially in the Niger Delta Region, oysters are naturally occurring seafood in the estuaries, coastal mangrove ecosystem and swampland [6,7], attached to the aerial prop roots of the mangrove trees and other objects that are stationary, including sea vessels much in their numbers. Oyster are common in riverine communities such as; Okrika, Abuloma, Andoni, Tema, Dabara, Buguma, Eleme Kalabari and Owoka all in Rivers State. Other states where oyster can be found include: Delta, Cross River and Bayelsa [1].

Oysters are more frequently implicated in illness/disease outbreaks than other bivalves, because during feeding they bio-accumulate, reserve and concentrate dissolved toxic substances or chemicals and microorganisms, including pathogens [3,8,9]. Oysters are eaten raw or cooked lightly in some tribes and cultures of the world; a practice that has given room for the transmission of harmful organisms to humans, resulting in mild gastroenteritis and life-threatening syndromes [5]. A number of authors have reported the presence of pathogens such as *Salmonella*, *Shigella*, *Vibrio*, and Hepatitis A from oyster in the past [2,10,11,12,13]. The unabated pollution of water body with both untreated and partially treated sewage is a major challenge, leading to seafood hazards [5].

Hazard analysis critical control point (HACCP) is pertinent in fishery sector because it evaluates, assess, implement and check the threat of contamination in food sectors. The HACCP concept which places emphasis more on safety of all ingredient and processing steps is a proactive systematic approach in controlling foodborne hazards [14], on the premises that safe products will result if processing is controlled. If critical control points (CCPs) are not properly controlled, the end-product may be unsafe for the public [15,16,17,18].

The study is therefore aimed at assessing the hazards associated with oyster from two communities in Rivers State and provides an insight at improving the safety of oyster through the application of HACCP concept in processing freshly harvested mangrove oysters.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Oyster Samples

The oyster samples were obtained from Kalio community in Okirika Local Government Area and Tari Ama in Abuloma Local Government Area both in Rivers State. The oyster samples were transported in sterile cooler to Microbiology Laboratory, University of Port Harcourt within 3 h for analysis.

### 2.2 Proximate Composition of Oyster

The proximate composition of the oyster was determined using the methods described by AOAC [19]. The parameters determined include: moisture, carbohydrate, crude protein, lipid, fiber and ash content.

### 2.3 Determination of the pH of Oyster Sample

Ten grams of oyster meat was homogenized in 20.00ml sterile distilled water using Benton electric blender (China). The pH value of homogenate was determined with a calibrated digital pH-meter (Hi-98107 pH, India) for both

treated and untreated oyster samples, as described previously [10]. The glass electrode was first standardized using buffer solutions of pH 7.0 for calibration. Ten milliliters (10 mL) of homogenized oyster sample were dispensed into a 100 mL beaker, and the electrode of the pH meter inserted into the sample, and after about 30s, the reading was recorded [19].

## 2.4 Processing

The oysters were washed with water, and 300 oysters were steamed for about 5min and shucked manually with a short thick blade knife about 5 cm (2.0 in) long. After shucking, they were washed, immersed in water in plastic bows and stored for sale and consumption at room temperature (28±2$^{o}$C) [10,20].

## 2.5 Hazard Analysis of Oyster Meat Samples

The analysis of oyster meat samples was evaluated using the hazard analysis critical control points (HACCP) concept. Hazard analyses were conducted following the method described by Bryan et al. [16] and Ehiri et al. [17]. Based on the observation and discussions made during collection of oysters from the local harvesters, during processing of oyster meat, a schematic diagram of processing of oyster was prepared. Fig. 1 shows the critical control points where samples were withdrawn for analysis. The steps were considered critical control point (CCP) as a loss of control could result in an increase in the microbial population and potential hazards. For instance, keeping the harvested oysters in dirty and contaminated baskets and containers will increase the microbial counts. Refrigeration is a CCP as it will check the growth of the accumulated microorganisms through their feeding habits. Steaming before shucking with sterile knife, use of contaminated water for washing and improper packaging are all CCPs. Twenty-five grams of the oyster sample were collected after each stage of processing for total aerobic plate counts.

## 2.6 Microbiological Analysis

Aerobic plate count (APC) was done by evaluating 25g of blended shucked oyster samples in 225ml 0.1N peptone water to obtain a 10-1 homogenate, after which serial dilutions were carried out from the homogenate and 0.1ml portions of appropriate dilutions were plated out using the spread-plate method, in triplicate on plate count agar (Biotech, India) supplemented with 1.0% Nacl. Coliform (*Escherichia coli*) were evaluated using the spread-plate method on pre-poured, surface-dried MacConkey agar (Biotech, India) while thiosulphate-citratebile-salt-sucrose agar (Biotech, India) was used to determine *Vibrio* on surface-dried plates using the spread-plate method. Cultured plates were incubated at 35ºC for 24-48h. Enumeration of colony forming units (CFUs) was done by counting representative colonies (30-300). Identification of bacterial isolates was based on cultural, physiological and biochemical characteristics of the isolates. Identification was confirmed using Cowan [21] and Bergey's Manual of Determinative Bacteriology [22].

## 2.7 Statistical Analysis

The one-way analysis of variance (ANOVA) was used to analyses obtained data and mean difference were determined at P =.05, using SPSS version 24.

# 3. RESULTS AND DISCUSSION

## 3.1 Proximate Composition

The proximate composition of oyster meat revealed the following: moisture (83.73%), protein (8.36%), lipid (1.28%), fiber (1.04%), carbohydrate (2.12%) and ash content (3.47%).

## 3.2 The pH of Raw Oysters

The pH of the oyster samples was essentially acidic. The pH of the conventionally processed oysters ranged from 3.2 to 6.8 while the pH of the oyster processed applying the hazard analysis concept ranged from 3.2 to 6.5.

## 3.3 Bacterial Profile of Raw and Processed Oyster Samples

The average aerobic plate count for Abuloma and Okrika were 5.69 and 6.98 log10CFU/g respectively while coliform count was 5.37 and 5.02 $\log_{10}$CFU/g respectively. The bacterial isolates obtained from oyster samples from Abuloma and Okrika are presented in Fig. 2. The predominant isolates obtained from Abuloma were: *Bacillus* spp. (30%), *Streptococcus* spp. (25%), and *Proteus mirabilis* (2%) while in Okrika the predominant isolates were *Bacillus* spp. (25%), *Streptococcus* spp. (16%), and *Pseudomonas* spp (4%).

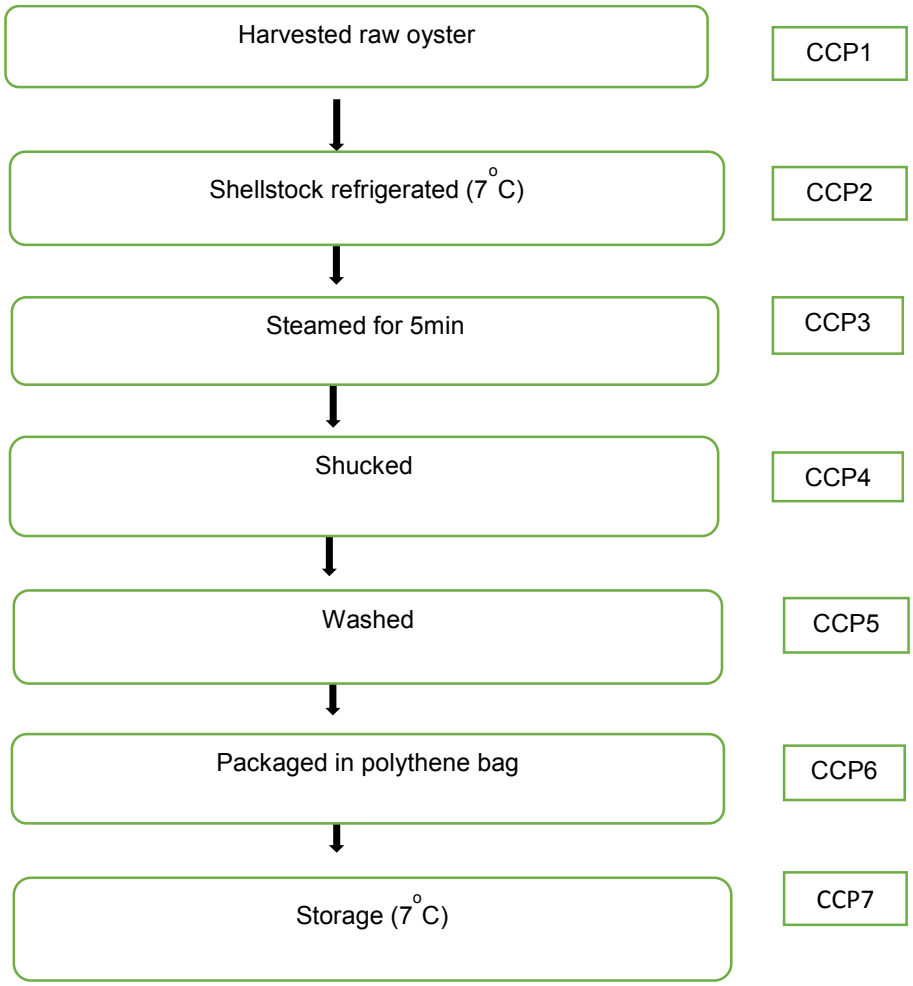


**Fig. 1. Critical control points (CCP) in the processing of oyster (*Crassostrea gasar*)**

The frequency of occurrence of bacterial isolates obtained from various critical control points of oyster processing is presented in Fig. 3. The value ranges from 7% (*Proteus mirabilis*) to 30% (*Bacillus* sp.) at CCP1; 17% (*Proteus mirabilis*) to 32% (*Streptococcus* spp.) at CCP2, 5% (*Bacillus* spp.) to 60% (*Staphylococcus aureus*) at CCP3, 28% (*Pseudomonas* spp.) to 60% (*Staphylococcus aureus*) at CCP4 and 7% (*Bacillus* spp.) to 63% (*Staphylococcus aureus*).

The total aerobic plate count from various critical control points ranged from 7.60 $\log_{10}$CFU/g to 3.20 $\log_{10}$CFU/g.

The proximate composition and intrinsic factors of any food determines the rate at which spoilage of the food occurs [14]. Oyster is a good source of protein and mineral (copper, iron, zinc) and vitamins such as: thiamin, riboflavin, ascorbic acid [2, 5]. The proximate composition of oyster meat sampled in this study shows that, oyster has high percentage moisture (83.73%) and protein content (8.36%) consistent with previous reports ranging from 62.18±0.39 to 80.15±0.34 and 12.87±0.37 to 18.66±0.81 respectively [23,24]. The ash and lipid contents are also comparable to a range of 2.08±0.02 to 5.03±0.22 and 2.16±0.05 to 3.73±0.18 respectively [23,24]. Crude fiber content (1.04%) was also the lowest occurring of all the components in previous reports by Efiuvwevwere and Amadi [5] and Ukwo et al. [24]. The high moisture and protein contents combined with storage temperature (28 ± 2°C) and conditions, chemical composition, level and types of microbial load and oxidative rancidity of the lipid (1.28%) have a way of influencing the microbiological quality and storage stability of seafood [5,25,26,27].

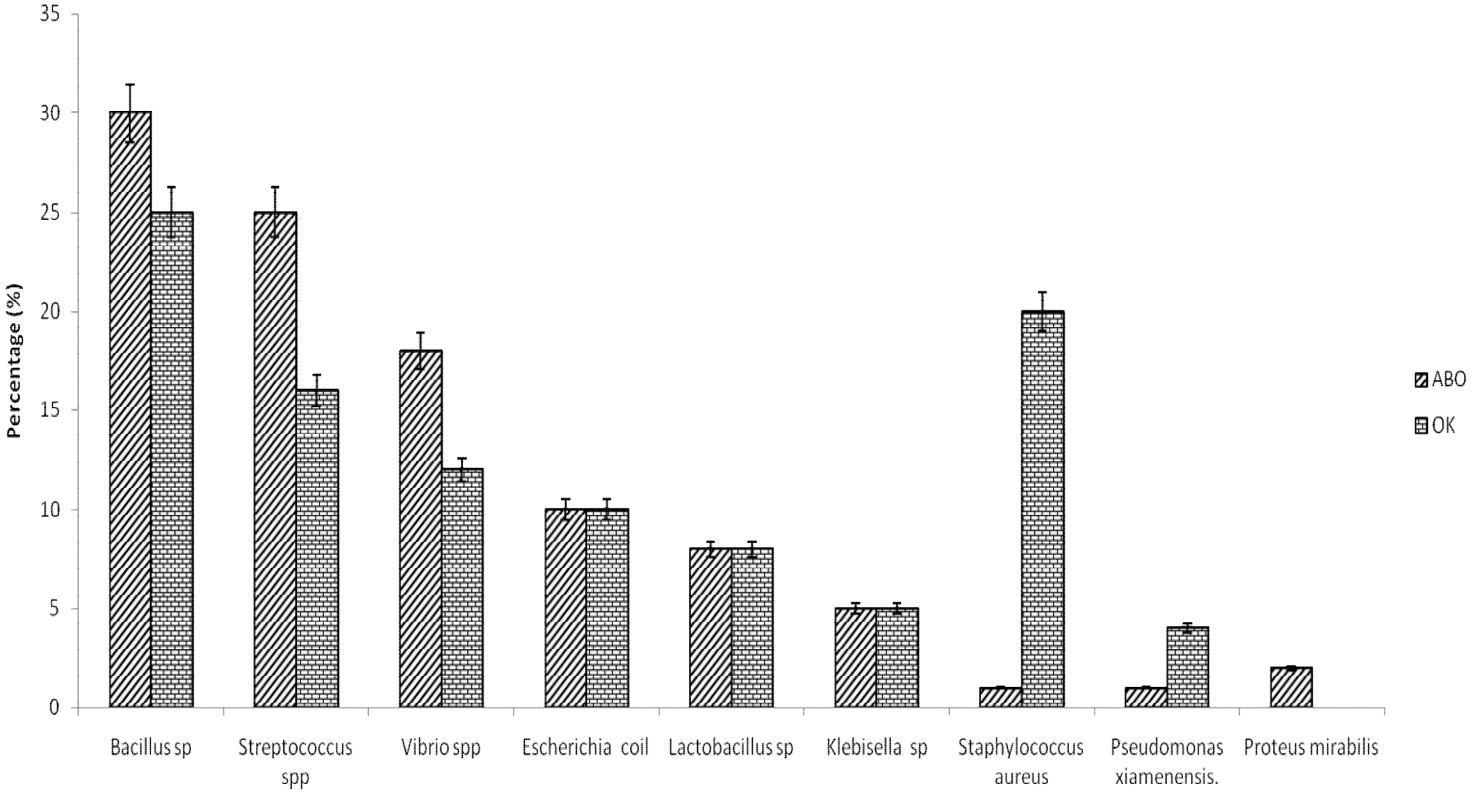


**Fig. 2. Frequency of occurrence (%) of bacterial isolates in oyster meat (traditionally) obtained from different locations**
*Key: ABO = Abuloma, OK = Okrika*

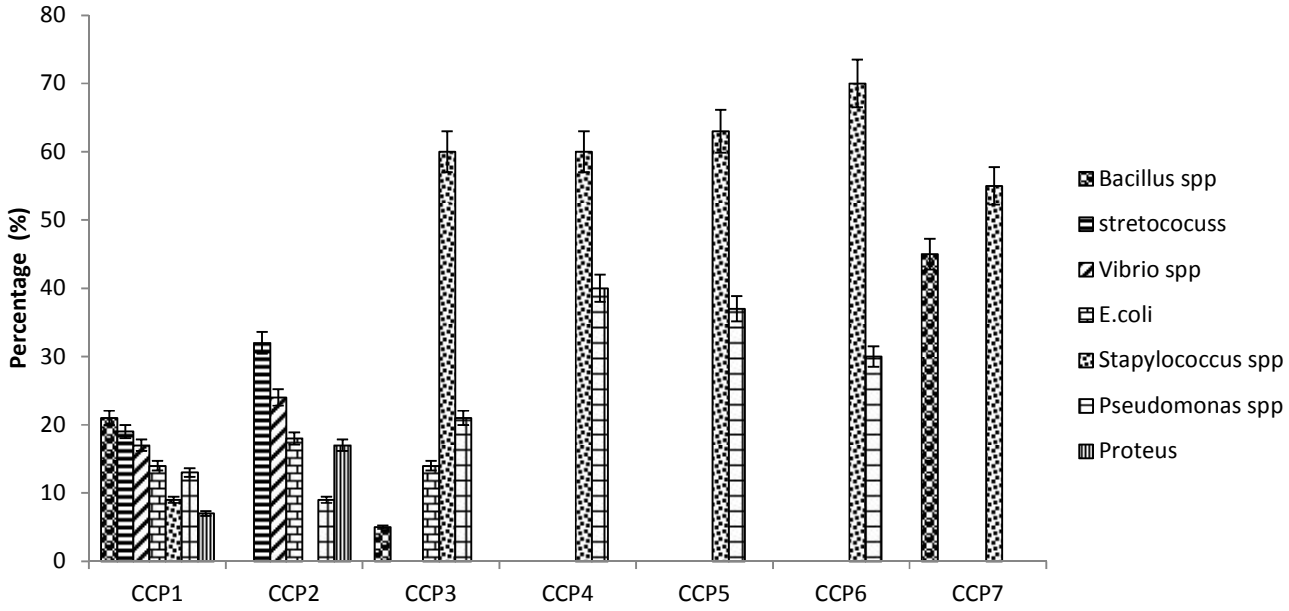


**Fig. 3. Percentage bacterial occurrence in oyster meat at various critical control points**

*CCP = Critical Control Points, CCP 1 = Harvested raw oyster, CCP 2= refrigerated 7oC, CCP 3 = steamed, CCP 4 = shucked oyster, CCP 5 = washing, CCP 6 = Packaged oyster, CCP7 = storage 7$^{o}$C*

Oyster meat harvested from Okrika had higher average aerobic plate count of 6.98 $log_{10}$CFU/g which exceeded the acceptable standard 5.70 $log_{10}$CFU/g ($5\times10^5$CFU/g) according to the International Commission for Microbiological Specification for Food [28]; while oyster meat harvested from Abuloma with average aerobic colony count 5.69log10CFU/g were within the marginal standard limit according to International Commission for Microbiological Specification for Food [28].The aerobic counts in this study are comparable with the report of 6.24 $log_{10}$CFU/g ($1.75\times10^6$CFU/g) by Omenwa et al. [29] in samples examined from ARAC, Buguma in South Eastern Nigeria but lower than the average count of 9.10 $log_{10}$CFU/g reported by Maduka and Ibe [30] in samples sold at Creek Road market in Port Harcourt. The high counts could be as a result of high anthropogenic activities in their estuary environment. *Escherichia coli* counts of oyster from both Abuloma and Okrika had counts lower than 8.37 $log_{10}$CFU/g reported by Maduka and Ibe [30]. The *E. coli* counts were far above the acceptable limits of 1.51 $log_{10}$CFU/g (230/100g) according to International Commission for Microbiological Specification for Food [28].

Nine bacteria genera, namely: *Bacillus* spp., *Pseudomonas* spp., *Streptococcus*, *Staphylococcus* spp., *Vibrio* spp., *Escherichia coli*, *Lactobacillus* spp., *Klebsiella* spp., and *Proteus mirabilis*, where isolated from the traditionally processed fresh oyster. A number of authors have reported the presence of the isolated bacteria in raw, boiled and roasted/smoked oyster samples [13,29,30,31]. *Bacillus* spp. were frequently isolated than any other bacteria in the oyster's samples examined. This is agreement with reports by Spillttstoesser et al. [32] that Bacillus spp. are the main spoilage organism in oyster meat. The persistence of Bacillus spp. in the oyster meat could be attributed to its ability to survive temperature that is high even during processing [33]. Abuloma has the highest number of heterogeneous bacteria which is indicative that the sites where they were harvested were highly contaminated with pathogenic and notable food spoilage bacteria. This may not be unconnected with the pollution of the water bodies with human and domestic waste as well as unhygienic practices of seafood handlers [5]. Oysters are filter feeder, therefore, reflect the types of microorganisms presents in their habitat [5,34].

The conventional method of processing oysters has a lot of unsafe and unhygienic practices such as non-temperature control, use of contaminated water for washing, improper packaging and storage and distribution methods. There is also the hazardous practice of storing shucked oyster meat with water from the source of harvest or contaminated water. Harvesting of oyster are usually done at night without refrigeration after harvest, thus they are stored in ambient temperature and conveyed from the harvest site in dirty sacks, baskets, exposing them to dust, sand, debris and other pollutants thus giving chances for the growth of mesophilic spoilage and pathogenic bacteria which poses health hazard to humans. The application of critical control points (CCP) on the oyster meat reduced the bacterial genera significantly (P = .05) from 7 to 2 genera. This has demonstrated that critical control points should never be taken for granted but be strictly applied. Among all the various results obtained from the CCPs. There should be functional regulatory bodies that ensures that fishing water are minimally polluted and within the recommended standard set by the WHO. Seafood from polluted water bodies should be destroyed or properly cooked. Basic fishing tools and cooling facilities should be made available for the local fishermen at the point of harvest and transportation to processors. Food processors should check for appearance of spoilage and hygiene quality of the harvested oysters and ensure a sustained process control and finished product inspection. The bacteria genera isolated at CCP1 (harvested raw oysters) is a reflection of the microorganisms present in the aquatic body where the oysters where harvested. Seven (7) bacteria genera; *Pseudomonas* spp., *Bacillus* spp., *Streptococcus* spp., *Vibrio* spp., *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis* where isolated at CCP1. Critical control point, 1 (CCP1) has the highest number of bacteria (7.6 $log_{10}$CFU/g) while CCP7 (storage at $7^oC$), has the lowest number of bacteria genera (two), (3.2 $log_{10}$CFU/g) *Bacillus* spp. and *Staphylococcus aureus*). There was significant reduction in bacterial genera at CCP2 following the refrigeration of the oyster at $7^oC$. Keeping/holding oyster meat for sale/supply at temperature more than $7^oC$, freeze-thawed or ambient temperature encourages the growth of microorganisms to hazardous level [9,18]. There was significant reduction in bacteria genera at CCP3 after steaming for 5 min as previously reported by Efiuvwevwere and Amadi [5]. Bacterial isolated from oyster sample were predominately Bacillus spp. which has the ability to survive in acidic environment at both room and refrigeration temperatures and at low pH hearse

environment produces spores that are resistant to moderate heating which later germinate at ambient temperature [9]. There was no significant reduction in bacteria genera at CCP4 and CCP5 after shucking and washing. Washing of oyster meat with potable water reduces or eliminates sources of faecal contamination, therefore sensitization and enlightenment campaign should be carried out and seafood processor should be educated on the adverse effect of using untreated or polluted water for processing of seafood. There was steady decrease of the microbial load and significant difference (P=.05) between CCP1 and CCP5, (7.6 $\log_{10}$CFU/g and 3.2 $\log_{10}$CFU/g). There was steady reduction of *Staphylococcus aureus* and *Bacillus* spp., and elimination of *Pseudomonas* spp., *Streptococcus* spp., *Vibrio* spp., *Escherichia coli*, and *Proteus mirabilis* of bacteria genera in the oyster meat during processing of oyster meat when the hazard analysis critical control points principles were applied. It is pertinent to state that only two genera; *Bacillus* spp. and *Staphylococcus* spp. were isolated from oyster meat subjected to HACCP principles at the final CCP.

## 4. CONCLUSION

Food safety is attainable if only the local oyster harvesters are educated on the right application of HACCP principles as a way of life. The critical control points (CCP) applied have a very positive effect on seafood, and can also reduce the number of health challenges common with consumption of this seafood. From this study, it is necessary for seafood possessors, vendor, consumers and the general public to be enlightened on the hazards associated with improper processed oysters. Application of HACCP concept during processing of oyster meat reduced the pathogenic organisms in the oyster meat as seen in this research work. Therefore, the application of HACCP in food industries should be highly encouraged especially in oyster meat processing. Oyster harvesters, processor and vendors, should be educated first on the benefits of HACCP so that the consumers can take the orientation serious. It is pertinent for government and appropriate authorities to provide the required basic infrastructure and service including; electricity for storage, potable water for washing and good road network for easy transportation of oyster products because all these have a way of affecting the oyster safety.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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