



Original Research Article

## Mortality and microbial diversity of raw, processed and storage of mangrove oysters (*Crassostrea gasar*)

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**Lawrence O. Amadi**

Department of Science  
Laboratory Technology, Ken  
Saro-Wiwa Polytechnic, P.M.B 20,  
Bori, Nigeria

Corresponding Author E-mail:  
[lawrenceamadi@ymail.com](mailto:lawrenceamadi@ymail.com)

Tel: +2348150610727

Mortality and microbial diversity of raw, processed and storage of mangrove oysters at ambient temperature were investigated. The mortality rates of raw (shell-stock) oysters were determined during depuration in tap water (TW) and brackish water (BW) microcosms for 14 days. Mortalities were observed on the 5th and 11th days and afterwards in TW and BW microcosms respectively. Thus, indicating the beneficial effects of depuration of mangrove oysters in BW than in TW microcosms. The microbial counts of raw, processed and storage of oyster meat samples were determined using standard microbiological methods. Aerobic plate counts (APCs) were  $1.36 \times 10^5$  and  $3.00 \times 10^3$  CFU/g for raw and processed oyster meats on day 0 ( $d_0$ ) respectively but increased markedly to  $1.55 \times 10^6$  CFU/g in the processed meat sample during storage for 24h. Fungi counts were  $3.6 \times 10^2$  and zero/no growth detected (NGD) CFU/g for raw and process oyster meats on  $d_0$  respectively but increased from 0-  $0.8 \times 10^2$  CFU/g in the processed meat samples at storage. Bacteria were more predominant in numbers and diversity than fungi. The most frequently isolated microflora from raw and processed and during the storage of oyster meat samples consists of *Bacillus*, *Pseudomonas*, *Vibrio*, *Proteus*, *Staphylococcus* others are *Aspergillus*, *Penicillium* and *Fusarium*. The most dominant genera during storage were *Bacillus* (20.8%) and *Pseudomonas* (16.7%); *Aspergillus* (52.3%) and *Penicillium* (45.4%). However, nondetectability of *E. coli* and *Acinetobacter* species following processing and storage underscores the criticality and importance of adequate processing prior to consumption of mangrove oysters as some of these organisms are not only potentially pathogenic but of public health significance. From this study, it is also advisable that oyster farmers should market their raw produce on/before 5 days for the depurated, and 24h for the processed meat samples to avoid serious postharvest economic losses.

**Key words:** Mangrove oyster, microcosms, microflora, mortality, processing, storage

### INTRODUCTION

Oyster is one of the popular and widely consumed food items which have historically been harvested from the wild in the intertidal areas and occurs either commercially or as a subsistence activity (Kaiser et al., 2001; Amadi and Efiuvwevwere, 2015). Wild bivalve populations also support important fisheries worldwide with capture

production exceeding 1.6 million tons in 2012 (FAO, 2014). These fisheries are economically important because unit price values for bivalves tend to exceed those for finfish and other invertebrates (Gosling, 2003; McDonald et al., 2015). Despite its universal acceptability and socio-economic incentives (Cao et al., 2009), oyster is rich in proteins,

minerals and vitamins (Adebayo-Tayo and Ogunjobi, 2008; Efiuvwevwere and Amadi, 2015b).

Being filter feeders, oysters are considered as potentially hazardous seafood (Forsythe, 2000; Lee et al., 2008; Lund, 2015) because of their tendency to bioaccumulate human pathogens and toxic metals (Burkhardt and Calci, 2000; NSSP, 2007; La Valley et al., 2009). It is being harvested from most natural productive waters, irrespective of the level of contamination and a major delicacy of many inhabitants of coastal communities in Nigeria. Cultured farms are at the experimental stages at the control of government ministries/agencies with no regulatory mechanisms in place and inappropriate disposal of raw and partially treated sewage are the major factors responsible for contamination and increasing incidence of oyster-associated illness following consumption. For this reasons many developed nations have set up regulatory agencies/organizations to monitor the microbiological quality of harvesting waters and/or bivalve flesh (NSSP, 2007; SARF, 2011).

Depuration, a process which reduces bioburden and toxic metals in the shellfish is not followed but sold at ambient temperature, conducive for the proliferation of mesophilic bacteria such as coliforms and most human pathogens (Hatha et al., 2005; Iwamoto et al., 2010; Amadi, 2014). Broad spectrum of microorganisms including *Escherishia coli*, *Staphylococcus aureus*, *Enterobacter*, *Bacillus*, *Vibrio*, *Pseudomonas aeruginosa*, *Proteus*, *Micrococcus*, *Lactobacillus* and *Acinetobacter* species as well as fungi such as *Apergillus niger*, *Penicillium* and *Fusarium* species have been identified in mangrove oysters (*Crassostrea gasar*) during postharvest depuration and storage (Adebayo-Tayo and Ogunjobi, 2008; Odu et al., 2012; Efiuwevwere and Amadi, 2015a,b). Understanding the dynamics of microbial communities of the oyster will provide insight into water pollution sources and estuarine health and may assist the development of new purification strategies for shellfish-borne human pathogens (La Valley et al., 2009). High perishability and short shelf-life are characteristics of oysters (Cao et al., 2009; Songsaeng et al., 2010; Montanhini and Montanhini, 2015) and primarily caused by microbial activity (Huss, 1995; Gram and Huss, 1996). Studies on microbiology of oysters have been fragmentary and limited to raw, sun-dried, oven-dried, smoked, and chemically preserved oysters (Adebayo-Tayo and Ogunjobi, 2008; Odu et al., 2012; Efiuwevwere and Amadi, 2015b) but with little or no information on the mortality and microbial population dynamics following processing and storage.

Gbolokiri creek which is the site of study is a brackish water/mangrove swamp ecosystem located at Rumuorlumeni, Obio/Akpor Local Government Area, Rivers State, Nigeria. The estuary consists of interconnecting creeks that link up the lower reaches of the New Calabar River (NCR). The salinity and temperature values of the estuary ranges between 9.40 and 23.50‰ and 24 and 32.5 °C at rainy and dry seasons (Amadi, 2014; Efiuvwevwere and Amadi, 2015a,b). There is a variety of economic

activities around the estuary such as fishing and farming and other establishments are educational institutions and cement industry most of which disposes human sewage, domestic and industrial wastes within and around the vicinity of the creek. Therefore, the identification of these organisms in raw, processed and during storage of mangrove oyster samples becomes of utmost relevance.

The present study was to evaluate the mortality and microbial diversity of raw, processed and storage of mangrove oysters at ambient temperature. Hence, knowledge of the shelf-life and postharvest deterioration processes of oysters can contribute to the reduction of losses, ensure microbial safety and expanded product distribution.

## MATERIALS AND METHODS

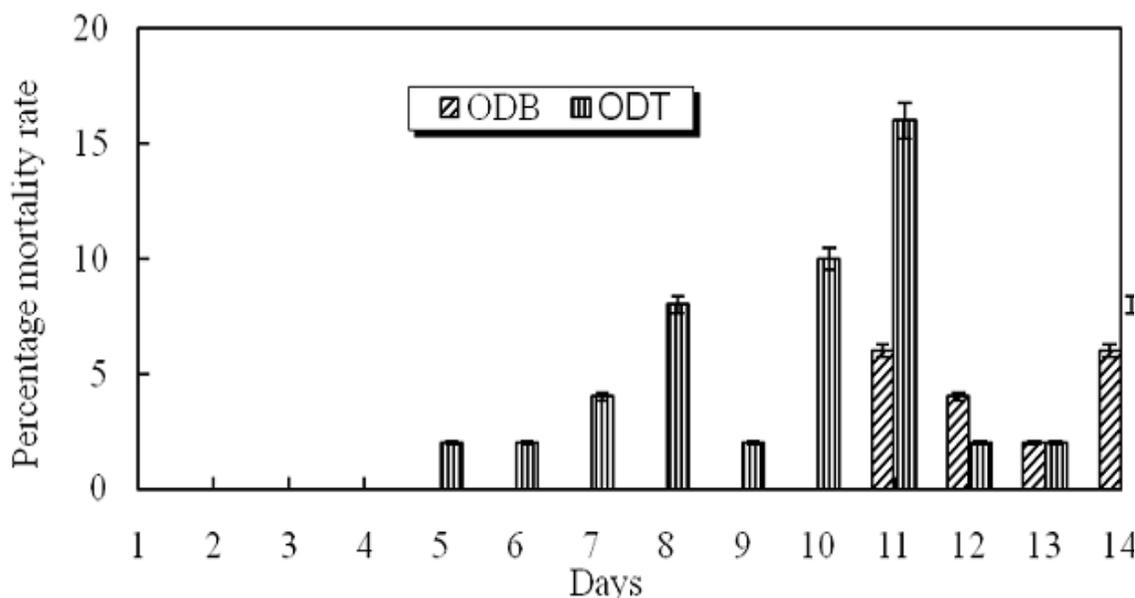
### Collection of raw oyster samples

Brackish water (BW) and raw mangrove oysters used in these experiments were part of a larger study on the bacteriological and physico-chemical parameters of microcosms used for depuration of mangrove oysters (Efiuvwevwere and Amadi, 2015a) obtained from the wild of Gbolokiri creek (between June, 2008 and May, 2009 on quarterly basis) of the NCR and tap water (TW) from the Departmental Laboratory served as control. BW and mangrove oysters were collected in sterile plastic container (20L) and polyethylene bags respectively and transported to the laboratory in less than 3hours. Fifty (50) raw shell-stock oysters of various sizes (1.70-11.20cm length) were thoroughly washed with TW to remove extraneous debris and excess mud on shells (Hunt et al., 1984) and randomly sampled to evaluate mortality in BW and TW respectively.

### Depuration and mortality of mangrove oysters

Two sterile rectangular plastic containers of 50x35x28cm dimension were used as tanks for depuration of oysters (*Crassostrea gasar*) in 7.5litres of BW and TW microcosms respectively. The water samples were changed at intervals of 24-hours as earlier reported (Chinivasagam, 1989; Obodai et al., 2010) for 14days. Activities monitored included shell opening and closure and rapidity of response to tactile stimulations or perturbations. All gapers or dead oysters with unclosed shells were removed and their number recorded. Mortality recorded was expressed as percentage of the test samples/populations (Dakar and Ekweozor, 2004). Thus,

$$\frac{\text{No. of dead oysters (x)}}{\text{Test sample}} \times 100$$



**Figure 1:**Percentage mortality rated of oyster depurated in BW and Tw microcosms

BW:Oyester dreuref in blackish warer(ODB)

TW:Oyester depurated in tap water(ODT)

Values represent thre mean of four determinations

Error bars represent the standsrd deviationa of four determination

### Preparation of oyster meat samples and storage at ambient temperature

Raw mangrove oysters (300) were randomly sampled and divided into two portions; one portion of the raw oysters (150) was hand-shucked for microbiological analyses whereas the other portion of 150 raw oysters was steamed at 100 °C for 5min (Efiuvwevwere and Izakpa, 2000) and shucked (i.e., the processing). The processed oyster meat sample was dipped into 300mL of sterile distilled water contained in 500mL sterile conical flask and sealed with aluminum foil before storage at (30±2°C) ambient temperature for 24hours. Thereafter, it was subjected to microbiological analyses. Following obvious deterioration and spoilage of samples after 24hours of storage, microbial analysis was discontinued.

### Microbiological analysis

Fungal counts were determined on Potato dextrose agar (PDA, Scharlau Chemie S.A. Spain) by plating (1.0ml) of appropriate dilution and incubation at 30 °C for 2-5days. Discrete colonies were thereafter aseptically picked and stained with lactophenol cotton blue on a microscopic slide and examined. Identification was by comparison of the observed macro and microscopic morphological characteristics (Harrigan and McCance, 1976; Samson and Reenen-Hoekstra, 1988). Aerobic/Total plate count (A/TPC) was determined by blending 25g of shucked oyster samples in 225mL 0.1N alkaline peptone water to obtain a

10<sup>-1</sup> homogenate. Further serial dilutions were made from the homogenate and 0.1 portions were spread-plated in duplicate on plate count agar (Scharlau Chemie S.A. Spain) supplemented with 1.0% NaCl (Dalgaard, 2000). Coliform counts (CC) including *Escherichia coli* (EC) counts were determined on pre-poured, surface-dried MacConkey agar (Oxoid Ltd., UK) while *Vibrio* counts (VC) were determined on surface-dried thiosulphate-citratebile-salt-sucrose agar (Lab M Ltd, UK) using spread-plate method and plates were incubated at 37°C for 24h respectively. Discrete colonies (30-300) were enumerated as colony forming units per gramme (CFU/G) and identification of bacterial isolates was carried out based on cultural, morphological and biochemical characteristics (Cowan and Steel, 1974; Sneath et al., 1986; Forbes et al., 2007).

### Statistical analysis

The analyses were carried out in duplicates on different occasions. The one way analysis of variance (ANOVA) was used to analyse obtained data for significance difference using SPSS Inc., 2007.

## RESULTS

### Mortality rates of raw (shell-stock) oysters during depuration

The percentage mortality rates of raw shell-stock oysters

**Table 1.** Microbial counts of raw, processed oysters and following 24hours of storage at ambient temperature

| Microbial group | Duration (day)       |                     |                      |
|-----------------|----------------------|---------------------|----------------------|
|                 | Raw                  | Day 0               | Day 1 (24h)          |
| <b>Bacteria</b> |                      | <b>Processed</b>    | <b>Storage</b>       |
| TPC             | 1.36x10 <sup>5</sup> | 3.0x10 <sup>3</sup> | 1.55x10 <sup>6</sup> |
| CC              | 7.18x10 <sup>3</sup> | 2.0x10 <sup>2</sup> | 1.15x10 <sup>5</sup> |
| VC              | 1.63x10 <sup>2</sup> | 1.0x10 <sup>1</sup> | 5.60x10 <sup>3</sup> |
| EC              | 1.55x10 <sup>2</sup> | NGD                 | NGD                  |
| <b>Fungi</b>    | 3.6x10 <sup>2</sup>  | NGD                 | 0.8x10 <sup>2</sup>  |

NGD = No growth detected. Each value represents mean of four determinations

**Table 2.** Microorganisms isolated from raw and processed oysters

| Duration (Days) | Oyster sample | Microorganism  |
|-----------------|---------------|--|
| 0               | Raw           | <b>Bacteria</b><br><i>Bacillus</i> spp., <i>Pseudomonas aeruginosa</i> ,<br><i>Proteus</i> spp., <i>Vibrio</i> spp., <i>Escherichia coli</i> ,<br><i>Staphylococcus aureus</i> , <i>Acinetobacter</i> sp.,<br><i>Micrococcus</i> sp., <i>Corynebacterium</i> sp.,<br><i>Lactobacillus</i> spp. |
|                 |               | <b>Fungi</b><br><i>Aspergillus niger</i> , <i>A. flavus</i> , <i>A. nidulans</i> ,<br><i>Penicillium</i> spp., <i>Fusarium</i> sp., <i>Rhodotorula</i> sp.   |
| 0               | Processed     | <i>Bacillus</i> spp., <i>Pseudomonas aeruginosa</i> ,<br><i>Proteus</i> spp., <i>Vibrio</i> spp., <i>Streptococcus</i> spp.,<br><i>Staphylococcus aureus</i>   |
|                 |               | <b>Fungi</b><br>-  |

Legend: - = Not isolated

depurated in BW and TW are shown in Figure 1. The initial mortalities of oysters in TW and BW microcosms occurred on days 5 and 11 respectively. The highest mortality rates of 16% and 6% were observed on day 11 in TW and BW respectively. The cumulative mortality rates of oysters during depuration from days 0-14 were 56% and 18% for TW and BW microcosms respectively, indicating the beneficial effects of depuration in BW microcosms than in TW.

### Microbial diversity in raw, processed oysters and following 24h storage

The highest bacterial count occurred during storage and the least count following processing whereas the highest fungal count was observed in raw oyster meats and NGD after processing, day 0 (Tables 1). The TPC value of 1.55x10<sup>6</sup> CFU/g during storage was the highest and the least was EC, no growth detected (NGD) following processing. A similar trend was observed with fungal counts except that raw oyster had the highest.

The raw oyster meat samples has the highest diversity of heterotrophic microflora; ten (10) genera of bacteria and four (4) of fungi, the lowest being the processed oysters

on day 0 with no fungus isolated (Table 2).

The most frequently isolated bacterial species are *Bacillus* species (20.8%) and *P. aeruginosa* (16.7%) whereas those of fungal species are *Penicillium* species (45.4%) and *Aspergillus flavus* (34.1%) (Table 3). The percentage (%) occurrence

of these microorganisms suggests their capacity to cause deterioration and spoilage of oyster meat samples during storage.

### DISCUSSION

The extended shelf-life of raw oysters following postharvest (Figure 1) may be attributed to several physiological adaptations to survive out of seawater, interrupted feeding activity and under temperature variations (Bartol et al., 1999; Kawabe et al., 2010; Montanhini and Montanhini, 2015). Although, some physico-chemical parameters were not measured in the present study, previous studies (Efiuvwevwere and Amadi, 2015a,b) indicate that depuration of oysters in BW and TW microcosms are characterized by slight fluctuations in temperature, pH, turbidity and salinity. The higher

**Table 3.** Microorganisms and their percentage (%) occurrence in processed oysters following 24 hours storage at ambient temperature

| Microorganisms             |                |                           |                |
|----------------------------|----------------|---------------------------|----------------|
| Bacteria                   | Percentage (%) | Fungi                     | Percentage (%) |
| <i>Bacillus</i> sp.        | 20.8           | <i>Penicillium</i> spp.   | 45.4           |
| <i>P. aeruginosa</i>       | 16.7           | <i>Aspergillus flavus</i> | 34.1           |
| <i>Vibrio</i> sp.          | 12.5           | <i>A. niger</i>           | 9.1            |
| <i>Streptococcus</i> sp.   | 12.5           | <i>A. nidulans</i>        | 9.1            |
| <i>S. aureus</i>           | 12.5           | <i>Fusarium</i> sp.       | 2.3            |
| <i>Proteus</i> sp.         | 8.3            |                           |                |
| <i>Lactobacillus</i> sp.   | 8.3            |                           |                |
| <i>Micrococcus</i> sp.     | 4.2            |                           |                |
| <i>Corynebacterium</i> sp. | 4.2            |                           |                |

mortality rates of oysters observed in TW microcosms may be due to poor adaptability and physiological activities. Conversely, depuration of oysters in BW (natural oyster habitat) was more favourable probably due to better adaptability, nutritional status obtained from extraction site and physiological activities, and may account for the extended period of 6days (Figure 1). This corroborates the findings of other workers (Songsaeng et al., 2010; Efiuvwevwere and Amadi, 2015a,b) who used artificial medium simulating BW microcosm to preserve and enhance the microbial safety of oysters.

The high incidence of TPC in raw oyster may be attributed to the environment and microniches to which they were exposed which enhances proliferation (Fraizer and Westhoff, 2005; La valley et al., 2009). Such increases in microbial counts (between 5.0-6.7 log CFU/g) and diversity has been earlier reported in raw oysters prior to storage (Green and Barnes, 2010; Fernandez-Piquer et al., 2012; Chen et al., 2013). In contrast, the sharp decrease in microbial counts following processing may not be unconnected to their susceptibility to heat processing treatments (Table 1) while the apparent increase with storage (6.0 log CFU/g) suggests sub-lethal effect and microbial recovery in food ecosystems (Efiuvwevwere and Amadi, 2015b). Based on the European Council Directive 93/493 EEC (FAO, 1993) of critical value of  $10^5$ CFU/g APCs in cooked shellfish, the processed oyster on day 1 of storage produced unpleasant odour and other offensive spoilage characteristics and so considered microbiologically unsafe. Moreover, total aerobic mesophilic counts between 6.0 and 8.0 log CFU/g has been previously reported to promote putrid odour, loss of quality and spoilage in seafoods (Huss, 1995; Madigan et al., 2014). These changes are also directly related to deterioration, particularly odour which is a major sensory quality attribute that best determines quality and freshness (Cao et al., 2009; Montanhini and Montanhini, 2015).

The absence of *E. coli* and *Acinetobacter* species (Table 2 and 3) following processing and storage is suggestive of their sensitivity to heat treatment (Eley, 1996) whereas their presence in raw oysters with other coliforms is indicative of anthropogenic and/or faecal contamination to

the mangrove ecosystem (Craig et al., 2004; Mignani et al., 2013; Ghaderpour et al., 2014). In contrast, the detection of *Bacillus*, *Streptococcus*, *Staphylococcus* and *Vibrio* species (Table 3) is indicative of their relative heat tolerance and/or protective effects of certain components of oysters such as protein, fat and carbohydrate (Jay, 2000; Selecky et al., 2007; Efiuvwevwere and Amadi, 2015b). Conversely, isolation of *S. aureus* in oysters may be attributed to lack of hygiene in the postharvest handling process (Bennett et al., 2013) or post-processing contamination. The high incidence of *Bacillus* and *Pseudomonas* species during storage suggests their capacity to cause rapid deterioration and spoilage of oyster meat samples and could be possible indicators for future shelf-life studies. Although, the predominance of bacteria in mangrove oysters have captured the attention of many researchers due to their virulence and pathogenicity, this does not preclude the presence of fungi. The present study revealed that those mycoflora isolated from mangrove oysters (Table 2 and 3) are similar to those earlier reported (Adebayo-Tayo and Ogunjobi, 2008; Obodai et al., 2010). Hence, knowledge of the postharvest deterioration process of oysters can contribute to the reduction of losses and expanded distribution of product.

## Conclusions

The mortality rate of raw (shell-stock) oysters was higher in TW microcosm than in BW microcosm with an extended shelf-life of six days. Processing, dramatically decreased microbial counts but with apparent increase during storage to APC of  $10^6$  CFU g<sup>-1</sup> and onset of deterioration and spoilage. The elimination of *E. coli* and *Acinetobacter* species suggests the importance of heat processing treatment before consumption of oysters. Results from this study should assist regional risk managers, producers and traders in developing policy guidelines on the storage of oysters prior to consumption.

## Conflict of Interests

The authors declare that there is no conflict of interests

regarding the publication of the paper

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