AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 11 No. 2
March 2011
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

MICROBIOLOGICAL QUALITY OF RAW AND PROCESSED FARM-REARED PERIWINKLES FROM BRACKISH WATER EARTHEN POND BUGUMA, NIGERIA

Omenwa VC[1]*, Ansa EJ[1], Agokei OE[1], Uka A[1] and OS George[1]

Veronica Omenwa

*Corresponding author email: marylin1308@yahoo.com

[1]African Regional Aquaculture Centre, P.M.B. 5122, Port Harcourt, Nigeria.

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 11 No. 2
March 2011
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

ABSTRACT

The microbiological quality of raw and processed periwinkles obtained from brackish water earthen pond of the African Regional Aquaculture Centre, Buguma, Rivers State, Nigeria was studied. The samples were harvested at exactly 11 am on a Monday morning, at high tide and water temperature of about 29°C. Ninety samples were analyzed and used for the study, which comprised the enumeration of indicator organisms and other pathogens as well as their total counts. Total bacterial counts of the samples from boiled periwinkle meat, boiled shell-on periwinkles and raw periwinkle meat were <10, 2.32 – 2.41 x 10^6, and 1.65 – 1.86 x 10^{6-} cfu/g, respectively. The boiled shell-on periwinkle sample had the highest level of microbial growth. The result of these microbiological examinations of boiled shell-on, raw and boiled periwinkle meat (without shell) showed that the boiled shell-on and raw periwinkle meat contained unacceptable levels of bacteria with a mean total bacterial count of 2.37 x 10^6 and 1.77 x 10^6 cfu/g, respectively. On the contrary the processed (boiled) periwinkle meat contained a total plate count of <10 cfu/g. The organisms isolated from all the periwinkle samples included *Salmonella paratyphi*, *Escherichia coli*, *Proteus vulgaris*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Erwinia amylovora* and *Serratia maracescense*. Indicator organisms especially *Salmonella paratyphi* and *Aerobacter aerogenes* seemed to be more common in the isolated samples with the highest number among the isolated bacteria especially in the boiled shell-on periwinkle and raw periwinkle meat. Some psychotropic organisms such as *Erwinia amylovora* and *Serratia maracescense* were also isolated although *Serratia maracescence* and *Proteus vulgaris* were the least encountered in all the samples analysed. The fungi isolated from raw periwinkle were *Fusarium* species *and Sacharomyces cerevisiae* with a fungi load of <10 cfu/g for both samples. There was no record of any fungal load in the boiled periwinkle meat. The isolation of indicator organisms as well as other pathogens from the boiled shell on periwinkles and raw periwinkle meat should be a source of concern to the consumers especially in South/South Nigeria where they are mostly cooked with the shell on, or inadequately cooked because of some perceived medical and nutritive values.

Key words: *Tympanotonus fuscatus*, Bacteria, Microbiological Quality, Fungi

INTRODUCTION

Periwinkles are marine mollusks that are represented in mangrove swamps, lagoons and estuaries by two genera *Tympanotonus* and *Pachymelania* [1].T*ympanotonus fuscatus* are shellfish dominantly found in brackish waters of the riverine areas of Nigeria, where they are highly prolific. This feature had made them a cheap source of protein in many homes when compared to other conventional protein sources [2]. They are also transported to many non-riverine towns and cities, where they are used to prepare various palatable dishes in hotels and restaurants, across the country, Nigeria. The best method to process periwinkles before consumption differs among the populace because of one reason or the other. Some people believe that periwinkles should be thoroughly washed, its pointed end cut off and then cooked with its shell because of its perceived medical and nutritive value while some other people believe that the shell should be removed and the meat washed thoroughly before cooking. However, studies on the microbiological quality of shell fishes have shown that they harbor many pathogenic microorganisms, which had been implicated in the outbreaks of food-borne diseases in many parts of the world [3]. Preparation of this shellfish is especially important in the Southern states where periwinkles are processed mainly by boiling with or without their shell. Most times, the accumulation and concentration of pathogenic microorganisms and other toxic materials are usually from untreated human waste and industrial effluents that find their way into the water bodies that are inhabited by these shellfishes [4, 5]. Hence, there is need to create awareness to the public on the health hazards of cooking shell-on periwinkle or consuming inadequately cooked periwinkle meat so as to prevent the ingestion of pathogenic microorganisms.. The objective of this study was, therefore, to evaluate the microbiological quality of boiled shell-on periwinkles, raw periwinkle meat and boiled periwinkle meat (without the shell) so as to determine a safe and healthy means for its processing and consumption.

MATERIALS AND METHODS

Collection of samples: mature periwinkles of mean sizes ranging from 41.69-46.85 mm in length and 4.52-5.33 g in weight were harvested from an earthen pond of the Brackish Water Research Station, Buguma, Rivers State, Nigeria, where they are reared until they reach certain sizes before harvest. Ninety pieces of the periwinkle samples were collected and stored in isothermic boxes containing dry ice until their arrival (within 6 hours) at the Microbiology Laboratory of the Federal University of Technology, Akure, Ondo State, Nigeria. The periwinkles were aseptically shared into three groups of thirty (30) each.

Preparation of samples [6]: the first group, sample ‘A’ was aseptically scrubbed and washed thoroughly under running water and the pointed ends were cut off. This is the periwinkle sample with shells. The second group, sample ‘B’ was also thoroughly scrubbed and washed and the meat aseptically extracted. For the third group, sample ‘C’, the meat was aseptically extracted and both samples ‘A’ and ‘C’ were processed by boiling under laboratory conditions at 100 °C for 5 min.

MICROBIOLOGICAL ANALYSIS [7]

The microbiological analyses were carried out in triplicate. Each sample was blended with 0.5% sterile peptone water. Standard plate counts were prepared from 6-fold dilutions in plate count agar for total bacteria count, *Salmonella - Shigella* agar and xylose-lysine deoxycholate agar, both for Salmonella and Shigella counts, sulfate tryptose broth and *Escherichia coli* broth for *E. coli* and *Aeromonas aerogenes*, mannitol salt agar for *Staphylococcus*, thiosulfate-citrate-bile salts-sucrose (TCBS) agar for *Vibrio* spp. and gluconate medium for *Pseudomonas* species. After incubation at 37 – 45.5 °C for 24 – 48 hours depending on the organisms being isolated, distinct colonies were selected randomly and characterized using various cultural, morphological and biochemical tests such as sugar fermentation tests, gram stain, catalase, starch hydrolysis, motility, spore stain, indole, oxidase, coagulase, oxidative fermentation and methyl red voges proskauer medium. The yeast – mold plates were prepared in Potato Dextrose Agar and incubated at 28 °C $\pm$ 1 °C for 72 hours. The bacteria, mold and yeast colonies and isolates were identified with the criteria of Holt *et al.* [8], Barnet *et al.* [9] and Van Rij [10], respectively.

Statistical Analysis

Differences between means were assessed using student's T test while the levels of significance of the data were calculated using analysis of variance according to Duncan [11].

RESULTS

Table 1 shows the levels of bacterial load in the raw and processed periwinkle samples. Total bacterial populations of the samples from boiled periwinkle meat, boiled shell-on periwinkle and raw periwinkle meat were <10, 2.32 – 2.41 x 10^6, and 1.65 – 1.86 x 10^6 cfu/g, respectively. The boiled shell-on periwinkle samples had the highest level of bacterial contamination. This is in the range of a study [12], which also reported total bacterial populations of fresh periwinkles to be 1.30 x 10^5 – 1.20 x 10^8cfu/g. This study also isolated *E.coli, Pseudomonas aeruginosa* and *Salmonella paratyphi* in the fresh periwinkle analyzed [12]. Even though the levels of microbial load in the raw periwinkle meat were less than those in the shell-on periwinkle, they were still unacceptable for human consumption as stipulated by the "International Commission on Microbiological Specifications for Foods" [13], which says that the maximum microbial count in shell fishes should not exceed the acceptable limit of 1 x 10^5 cfu/g for the safety of the consumer. The boiled periwinkle meat had a total bacterial load of less than ten (<10 cfu/g) with no fungal growth. *Fusarium* species and *Saccharomyces cerevisiae* were the fungi isolated from the shell-on periwinkle and raw periwinkle meat with a fungi load of less than ten (<10 cfu/g).

DISCUSSION

The result showed that the microorganisms in the boiled periwinkle meat without shell were significantly lower than those in the boiled shell on periwinkles ($p<0.05$).

The result of the boiled periwinkle meat showed that adequate boiling of the periwinkle meat helped to drastically reduce the microbial load of the periwinkle samples. The indicator and pathogenic organisms isolated (Table 2) such as *Salmonella paratyphi, E.coli, Aerobacter aerogenes, Pseudomonas aeruginosa* and coagulase positive *Staphylococcus aureus* were encountered more in the boiled shell-on periwinkle samples, which is an indication of contamination of the periwinkle shells from the polluted cultivation water [4,5]. This result is in consonance with previously reported works [14, 15], which stated that the level of pollution of the cultivation waters determines the level of contamination of shellfish. This is because the shells of periwinkles are capable of harboring pathogenic microorganisms. This should be a source of concern to consumers especially in the riverine states of Nigeria, where people prefer to cook the periwinkles with the shell and in some cases these shells are not properly washed before processing. The present results show that boiling could not entirely eliminate these microorganisms.

The raw periwinkle meat also contained some of the pathogenic organisms although at a minimal level with some psychotropic organisms such as *Erwinia amylovora* and *Serratia maracescense* (Table 2). *Aerobacter aerogenes* and *Salmonella paratyphi* had the highest rate of occurrence while *Serratia maracescence* and *Proteus vulgaris* were the least encountered.

Activities that bring about pollution such as bathing, defecation by the local population coupled with effluents from industries that eventually find their way into the water might have brought about the high level of contamination observed in this study, especially, the isolation of enteric microorganisms whose sole source is always faecal waste or sewage. The results of this study also agree with Adebayo-Tayo *et al.* [14] who observed unacceptable levels of bacterial contaminant in two periwinkle samples from a freshwater and brackish water environment (Southern, Nigeria). Jay [4] also reported that marine products are usually contaminated by those bacteria that originate from the contamination of the water body with human residues and those bacteria that are naturally present in the water environment. All the microorganisms isolated have health implications to man especially the enteric organisms *Aeromonas aerogenes* is implicated in both pediatric and adult populations where they cause acute gastroenteritis and bacteremia in persons with underlying hematological malignancies or hepatic dysfunctions [16]. *Staphylococcus aureus i*s a major cause of cerebrospinal fluid shunts in children. *Salmonella paratyphi* causes food poisoning (Salmonellosis), a major cause of death in United States between the years 1990 - 1998 [17]. *Escherichia coli* cause infantile diarrhea and newborn meningitis. *Pseudomonas aeruginosa* commonly thrive in burns, wounds and some blood infections. Therefore, pseudomonas may have occurred due to bathing of the locals with open wounds or other infections.

This study revealed that periwinkle, *Tympanotonus fuscatus* even though is a cheap source of protein, has the tendency of harboring pathogenic microorganisms especially those that are relevant to human health, mainly because of the unsanitary condition of the cultivation water and this becomes more alarming when the periwinkles are boiled with their shells or when they are inadequately heat-processed.

CONCLUSION

The microbiological examination of raw and processed periwinkle samples revealed that the boiled shell-on periwinkles are more contaminated than the raw periwinkle meat, while the boiled periwinkle meat (without shell) have reduced microbial load. These results indicate that the shell-on and raw periwinkle meat contain unacceptable levels of microbial load. Considering the health implications of high microbial load in foods, proper attention should, therefore, be paid to the safety of processed periwinkles through the quality of water used for the cultivation. Other safety measures could include proper handling, use of adequate processing procedures such as proper heating and shell removal as well as the use of depuration (controlled purification) method in order to reduce the level of pathogenic microorganisms in the shell fish.

Table 1:Mean Total Microbial Count of the Raw and Processed Periwinkles

Samples	Total bacterial count (cfu/g) Mean ± SD	Total fungal count (cfu/g) Mean ± SD
SOP	$2.37 \times 10^6 \pm 4.51 \times 10^4$	2.67 ± 0.58
RPM	$1.77 \times 10^6 \pm 1.07 \times 10^5$	1.33 ± 0.58
BPM	2.0 ± 1.0	-

The mean values are significantly different ($p<0.05$)

Key: SOP = Shell-On Periwinkles (boiled)
RPM = Raw Periwinkle Meat (without shell, not boiled)
BPM = Boiled periwinkle meat (without shell)
- = No growth.

Table 2:Microbial Isolates from Raw and Processed Periwinkles

Samples	Microbial Isolates
SOP	*Salmonella Paratyphi, Escherichia coli, Proteus vulgaris, Aerobater aerogens, Pseudomonas aeruginosa, Staphylococcus aureus, Fusarium sp. and Saccharomyces cerevisiae*
RPM	*Salmonella paratyphi, Staphylococcus aureus, Erwinia amylovora, serratia maracescense, Aerobacter aerogenes and saccharomyces cerevisiae*
BPM	-

Key:

SOP = Shell-on Periwinkles (boiled)

RPM = Raw Periwinkle Meat (without shell, not boiled)

BPM = Boiled periwinkle meat (without shell)

- = No growth.

REFERENCES

1. **Buchaan JB** Marine mollusks of Gold Coast of West African. *Journal of West African Science Association*. 1954; **7**: 30 -45.

2. **Bassey IO and AA Ayuk** Effects of Dietary Supplementation of Periwinkles *(Pachymelania aurita)* Flesh on Meat Quality of Broilers Fed Palm Kernel Cake Based Diets. *Journal of Food Agriculture and Environment.* 2007; **5 (3 and 4)**: 330-333.

3. **Ukpong E and O Utuk** Microbiological Quality of *Egaria radiata* in Cross River. Book of Abstracts. *Nig. Insti. Food Sci. Technol.*, 1992; pp 15-16.

4. **Jay JM ed.** Modern Food Microbiology. Aspen Publishers, Maryland USA. 2000: 620.

5. **Montgomery M and M Needselman** Board of Reagent of the University of Wisconsin System (BRUNS). The welfare of Toxic contaminants in fresh water fish, 1997; 2.

6. **APHA.** "Recommended Procedure for the Examination of seawater and shellfish". 4th edn. Amer. Pub. Health Assn. Washing, D.C APHA. 1990.

7. **APHA.** Compendium of Methods for the Microbiological Examination of Foods. 3rd edn, ed. Carl vanderzant, Don. F. Spilttstoesser. Am. Pub. Health Assn., Washington, D.C. 1992.

8. **Holt JG, Krieg NR, Sneath PH, Stanley JJ and ST Williams** Bergey manual of Determinative Bacteriology. Wilkins Published, Baltimore. (1994).

9. **Barnet JA, Payne RW and D Yarrow** Yeast Characterization and Identification. 2nd edn. Cambridge University Press, New York. 1983.

10. **Van Rij K** The yeast. A Taxonomic Study. 3rd edn. Elsevier Amsterdam, 1984; 55-57.

11. **Ducan DB** Multiple Range as Multiple F Test.Biometrics.1955; **2**:1-42.

12. **Adebayo – Tayo BC and AA Ogunjobi** Comparative Effects of Oven Drying and Sun Drying on the Microbiological Proximate Nutrient and Mineral Composition of *Tympanotonus* spp. and *Crassostrea* spp. *EJEAFChe* 2008;**7(4)**: 2856 – 2862.

13. **International Commission on Microbiological Specifications for Foods, ICMSF** "Microorganisms in foods, 1. Their Significance and Methods of Enumeration". 2nd ed., Intern. Comm. On Microbiolog. Spec. for foods. Univ. of Toronto Press, Toronto, Ontario, Canada. 1988.

14. **Adebayo – Tayo BC, Onilude AA, Ogunyobi AA and DO Adejoye** Bacteriological and Proximate Analysis of Periwinkles from Two Different Creeks in Nigeria. *World Applied Sciences Journal.* 2006; **1 (2):** 87-91.

15. **Ekanem EO and GO Adegoke** Bacteriological study of West African Clam (*Egeria radiata Lamarck*) and their overlying waters. *Food microbiol*. 1995; **12**: 381 -385.

16. **Janda JM and PS Duffey** Mesophilic Aeromonads in Human Disease: Current Taxonomy Laboratory Identification and Infectious Disease Spectrum. Rev: Infections Disease. 1975; **10 (5)**: 980 -973055195 (P, S E, B) cited 29.

17. **Brands DA, Inman AE, Gerba CP, Marei CJ, Billington SJ, Saif LA, Levine JF and LA Jones** Prevalence of Salmonella spp. in Oysters in the United States. *Journal of Applied and Environmental Microbiology*. 2005; **71 (2**): 893 -897.