

Comparative Microbial Evaluation of Two Edible Seafood *P. palludosa* (Apple Snail) and *E. radiata* (Clam) to Ascertain their Consumption Safety

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Abstract

Microbial evaluation of two edible seafood, *Pomacea palludosa* (apple snail) and *Ergeria radiata* (clam) were undertaken in this study to ascertain its consumption safety. These sea-food were processed as either fresh sun-dried or cooked oven-dried samples. Results revealed that the bacteria load in fresh samples of *E. Radiata* (2.20×10^6 - TNT CFU/ml) was significantly ($p < 0.05$) higher compared with *P. palludosa* (6.10×10^3 - 3.30×10^8 CFU/ml). The coliform count was also significantly ($p < 0.05$) higher in *E. radiata* (4-10 coliform/100 ml) than in *P. palludosa* (3-6 coliform/100 ml). Cooking significantly ($p < 0.05$) reduced the bacteria load and coliforms in both samples. In *P. palludosa*, a total of 26 bacteria were isolated: *Staphylococcus aureus* and *Streptococcus pneumoniae* were most frequent, a total of 20 bacteria were isolated from *E. radiata*, *Vibrio spp.* and *E. coli* being most frequent. The three fungi isolates from fresh *E. radiata* and *P. palludosa* were completely eliminated by cooking. In conclusion, fresh samples of clam and apple snail contain huge microbial load, hence adequate processing and proper cooking is needed before they are consumed. Nevertheless, the vast microbial loads in these species of sea-food could serve as a ready source of microbes in some processing industries.

Keywords: *P. palludosa*; *E. radiata*; Bacteria; Fungi; Coliforms

Introduction

Pomacea palludosa (apple snail) are tropical fresh water snail from the family *ampullaridae* (sometimes referred to as *pilidae*), while clams (*Ergeria radiata*) are bivalves mollusks with two shells that provide protection to the soft body. There are over 15,000 different species of these seafood's worldwide [1,2].

These sea-food have long been the focus of nutritional studies. Nutritionists consider them as important sources of high quality protein, minerals, vitamin D and essential fatty acids including omega-3-fatty acids [3]. The omega-3 fatty acids are involved in the prevention of cardiovascular diseases [4]. Hence, the national nutrition and health programme (PNNS) in France recommends consumption of these seafood twice a week especially for people who have heart attacks [5,6].

Report by Ifon and Umoh [7] also indicates that *Ergeria radiata* from riverine areas in Nigeria is rich in protein and vital elements and their protein content compares reasonably well with values obtained from whole hens' eggs, this further justifies the consumption of these seafood's as cheap and good sources of animal proteins [4,8].

Nevertheless, these seafood's are harvested from muddy and contaminated rivers. *Ergeria radiata* on the other hand is found in big rivers with high rate of oil spills such as Ibeno, Calabar-Itu rivers etc, whereas *Pomacea palludosa* is found in fresh water streams devoid of the activities of oil companies. Microbial and environmental factors may play a role in determining the nutritional composition of these calcerous species. Reports on the microbial evaluation of *E. radiata* and *Pomacea palludosa* are however scanty.

It is therefore the aim of this study to undertake a comparative microbial evaluation of *Ergeria radiata* and *Pomacea palludosa* in order to ascertain their safety for consumption and the possible value of these edible sea food in processing industries.

Materials and Methods

Collection and preparation of *E. radiata* and *P. palludosa*

Samples of *Pomacea palludosa* used for this study were harvested

and bought from a riverine fresh water habitat at Idomi, Yakurr, Central Cross River State. Some were bought from a local market at Aningheje in Akampka Local Government Area of Cross River State. *Ergeria radiata* samples were freshly harvested from Calabar Itu bridge beach market in Akwa Ibom and Watt market in Calabar, Cross River State. We collected the fresh samples between the months of January to March, 2009.

Soon after collection, the samples were within hours conveyed to the Laboratory Biochemistry Department for processing. We washed the samples with clean tap water to remove sand and other particles. Each edible portion of the *Ergeria radiata* and *P. palludosa* were removed from their calcerous shells, for *E. radiata*, the edible portion was removed by making a bilateral incision to expose their content of the stomach which was flushed out with clean tap water and then dried and for *P. palludosa*, the apple shaped shell was cracked after steeping in hot water for 5 minutes and the edible portion removed. After removing the edible portions, the samples were washed, pooled together and divided into two portions, one portion remained fresh sun-dried until it was crispy and powdered. The other portion was cooked and oven dried at 60°C until it was crispy.

Microbial evaluation

Microbiological investigations were carried out in the biological sciences laboratory of the Faculty of Science, University of Calabar-Nigeria.

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Received August 05, 2014; **Accepted** October 25, 2014; **Published** October 29, 2014

Citation: Bassey SC, Ofem OE, Essien NM, Eteng MU (2014) Comparative Microbial Evaluation of Two Edible Seafood *P. palludosa* (Apple Snail) and *E. radiata* (Clam) to Ascertain their Consumption Safety. J Nutr Food Sci 4: 328. doi: 10.4172/2155-9600.1000328

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Reagents: The reagents were mainly BDH chemicals prepared as specified by Lennette et al. [9].

Preparation of media

The recipe for preparation of media used followed the methods described by Cheesbrough [10] in Biomerieux API, 1989[11].

Enumeration of aerobic heterotropic bacterial count (method of Holts, 1982)

Surface spreading technique was used to determine the total number of aerobic heterotropic bacteria present in the sample. Serial dilution of the samples were prepared from 10^{-1} to 10^{-10} and 0.1 ml of each dilution was plated onto MacConkey and nutrient agar containing 5 ng/ml of Nystatin to inhibit fungal growth. The plates were prepared in duplicates and incubated at 37°C for 24 hours before enumeration [12].

Enumeration of aerobic heterotrophic fungi (Hunter and Bennet, 1973)

The total numbers of fungi present in the samples were enumerated by viable plate count method using surface spreading techniques. Serial dilutions of 10^{-1} to 10^{-3} of the sample were made. 0.1 ml of each dilution was plated into malt extract agar containing 10% lactic acid per ml to inhibit bacterial growth. The plates were prepared in duplicates and incubated at 28°C for 72 hours before enumeration [13].

Viable count method

All plating and counts were done by the pour plate technique of Harrigan and McCance [14].

Calculation of viable count

Number of colonies = no. of colonies counted x dilution factor x plating factor

Purification and maintenance of bacterial and fungal isolates (Cowan and Steel, 1974)

The bacteria and fungi isolates were purified by repeated sub-culturing. Isolates were subjected to a series of transfers unto fresh media. The bacterial and fungal isolates were incubated at 37°C for 24 hours and 28°C for 72 hours respectively. Pure colonies of bacteria and fungi were maintained on slope of nutrient agar and malt extract agar slants respectively. The slants were stored in a refrigerator at 8°C until needed [15].

Characterization and identification of microbial isolates

The bacterial isolates were examined for colony morphology as well as for cell micro-morphology and biochemical characteristics according to the methods described by Gerhardt et al. [16]. Identification of the bacteria to the generic levels followed the scheme of Holt [12]. The fungi isolates were characterized based on the macroscopic and microscopic appearances. Their probable identities were determined according to Hunter and Bennette [13] and Biomerieux API (1989) identification schemes.

Statistical analysis

All data were analyzed using the statistical package for social sciences (SPSS) version 17.0 built by Microsoft Corporation, USA. The data were analyzed by one way ANOVA and significant ones followed with a post-hoc (LSD) test between groups. All data were expressed as mean \pm SEM and probability tested at 95% level of significant ($p < 0.05$).

Results

Total microbial count in fresh sun dried and cooked samples of *Pomecia palludosa* and *Ergeria radiata*. Table 1 presents the total microbial count in fresh and cooked samples of *Pomecia palludosa* and *Ergeria radiata*. The total microbial count for fresh sun dried *Pomecia palludosa* ranged from 6.10×10^3 to 3.30×10^8 CFU/ml, their coliform counts ranged from 3 to 6 coliform/100 ml respectively, values for cooked were 3.80×10^3 - 2.50×10^5 CFU/ml and 10-20 coliform/100 ml respectively. There was marked reduction in microbial load as a result of cooking. If not properly cooked pathogens especially spore formers could survive the cooking temperature.

The total microbial count for *Ergeria radiata* ranges from 2.20×10^6 CFU/ml to too numerous to count and 4-10 coliforms respectively. The values for cooked *Ergeria radiata* were 3.00×10^3 - 1.70×10^5 CFU/ml and zero coliforms. The microbial load and coliform counts were higher in the fresh *E. radiata* (indication of probability of pathogenicity) compared with *P. palludosa*.

Cooking was able to reduce the microbial load effectively and completely eliminated the coliform. Cooking could be an effective means of reducing or preventing infection from this aquatic fauna.

Frequency of microbial (bacterial) and fungi isolates in fresh and cooked samples of *E. radiata* and *Pomecia palludosa*

As shown in Table 2 for *P. palludosa* and *E. radiata* respectively are results of bacteria and fungi isolates in the samples.

The results show that *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyrogens*, *Serratia marcesseus*, *Escherichia coli*,

| Variable | Range value of total count (CFU/ml) | Range values of no. of coliform per 100 ml |
|--|---|--|
| <i>Pomecia palludosa</i> (fresh sun dried) | 6.10×10^3 - 3.30×10^8 | 3-6 coliform/100 ml |
| <i>Pomecia palludosa</i> (cooked) | 3.80×10^3 - 2.50×10^5 | 10-20 coliform/100 ml |
| <i>Ergeria radiata</i> (fresh sun dried) | 2.20×10^6 - TNT | 4-10 coliform/100 ml |
| <i>Ergeria radiata</i> (cooked) | 3.00×10^3 - 1.70×10^5 | 0 coliform/100 ml (no growth) |

Table 1: Total microbial count in fresh sun dried and cooked samples of *Pomecia palludosa* and *Ergeria radiata*.

| S.No | Microbial isolate | <i>Pomecia palludosa</i> | <i>Ergeria radiata</i> |
|-------|-----------------------------------|--------------------------|------------------------|
| 1 | <i>Staphylococcus aureus</i> | 7 (26) | 3 (17) |
| 2 | <i>Streptococcus pneumonia</i> | 6 (23) | 1 (6) |
| 3 | <i>Streptococcus pyrogens</i> | 4 (15) | 0 (0) |
| 4 | <i>Serratia marcessens</i> | 3 (12) | 2 (11) |
| 5 | <i>Micrococcus</i> | 1 (4) | 0 (0) |
| 6 | <i>Escherichia coli</i> | 3 (12) | 4 (22) |
| 7 | <i>Staphylococcus epidermidis</i> | 2 (8) | 0 (0) |
| 8 | <i>Bacillus spp</i> | 0 (0) | 2 (11) |
| 9 | <i>Vibro spp</i> | 0 (0) | 6 (33) |
| Total | | 26 (100) | 18 (100) |

Values are presented as frequency and percentages (in parenthesis) *Staphylococcus aureus* and *Streptococcus pneumonia* have higher frequency than other isolates. *Staphylococcus aureus*, *Streptococcus pneumonia*, *S. pyrogens* and *E. coli* have been implicated in pathogenicity.

Table 2: Frequency of microbial isolates (bacteria) in fresh *Pomecia palludosa*.

Staphylococcus epidermidis and *Micrococcus* were bacteria identified in *P. palludosa*. The corresponding fungi were *Sacchromyces cerevisiae*, *Aspergillus spp*, *penicillium spp*. The *Sacchromyces cerevisiae* was found in all samples while samples 1, 2, 5, 7 and 8 contain *Penicillium* and *Aspergillus* in addition. The rest contain only penicillin. It was observed that sample can survive in dry form for more than 4 months.

The probable bacteria and fungi isolates in *E. radiata* were *Bacillus spp.*, *Vibro spp.*, *Escherichia coli*, *Candida albicans*, *Streptococcus pneumonia* (scanty), *Sacchromyces cerevisiae* (yeast), *Serratia marscessens* (*scanty*) and *Streptococcus aureus*.

The total microbial frequencies of 26 and 20 were recorded for *P. palludosa* and *E. radiata* respectively. *Staphylococcus aureus* and *Streptococcus pneumonia* most in *P. palludosa*; both organisms alongside *E. coli* have been implicated in pathogenesis. The microbial isolates from fresh *E. radiata* shows higher frequencies of *Vibro spp.*, *E. coli*, *Streptococcus pneumonia*, *Streptococcus aureus* and *Bacillus spp*. Which have all been implicated in various pathogenic infections.

Effect of cooking on the frequencies of microbial loads of *P. palludosa* and *E. radiata*

Table 3 shows effect of cooking on frequency of microbial isolates, cooking drastically reduced the microbial load from a total of 26 in fresh to 3 in cooked samples of *P. palludosa* and from a total of 18 for fresh *E. radiata* to 4 in cooked samples of *E. radiata*.

Frequencies of fungi isolates in fresh *P. palludosa* and *E. radiata*

As shown in Table 4, the frequency of fungi isolates in fresh *P. palludosa* shows presence of three fungi (*S. cerevisiae* (yeast), *Aspergillus spp.* and *Penicillium spp.*) with *Saccharomyces cereviva* being most occurring. *Aspergillus* implicated in *Aspergillois*, a fungi infection responsible for fungi food poisoning. However, in *E. radiata*, two types of yeast isolates *S. cerevisiae* and *Candida albicans spp.* were identified. No fungi isolate were identified in cooked samples of both *E. radiata* and *P. palludosa*.

| SN | Microbial isolate | <i>Pomecia palludosa</i> | <i>Ergeria radiata</i> |
|-------|---------------------------|--------------------------|------------------------|
| 1 | <i>Bacillus spp</i> | 1 (33.33) | 1 (25) |
| 2 | <i>Salmonella</i> | 1 (33.33) | 0 (0) |
| 3 | <i>Streptococcus spp</i> | 1 (33.33) | 1 (25) |
| 4 | <i>Staphylococcus spp</i> | 0 (0) | 1 (25) |
| 5 | <i>E. coli</i> | 0 (0) | 1 (25) |
| Total | | 3 (100) | 4 (100) |

Values are presented as frequency and percentages (in parenthesis)
Salmonella has been implicated in typhoid fever and therefore unsafe in cooked. Requires cooking over a long period of time.

Table 3: Frequency of microbial isolates (bacteria) in cooked *Pomecia palludosa*.

| SN | Microbial isolate | <i>Pomecia palludosa</i> | <i>Ergeria radiata</i> |
|-------|---------------------------------------|--------------------------|------------------------|
| 1 | <i>Saccharomyces cereviva</i> (yeast) | 10 (37) | 4 (50) |
| 2 | <i>Aspergillus spp</i> | 8 (27) | 0 (0) |
| 3 | <i>Penicillin spp</i> | 9 (33) | 0 (0) |
| 4 | <i>Candida spp</i> (yeast) | 0 (0) | 4 (50) |
| Total | | 27 (100) | 8 (100) |

Values are presented as frequency and percentages (in parenthesis)
 -*Aspergillus* is implicated in *Aspergillois* a fungal infection through food
 -*Candida albicans* has been implicated in candidiasis common in women.

Table 4: Frequency of fungi in fresh *Pomecia palludosa* and *Ergeria radiata*.

Discussion

study on comparative microbial evaluation of *Ergeria radiate* (clams) and *Pomecia palludosa* (gastropods) delicacies and effects of processing methods reveals that edible fresh food samples of *E. radiate* and *P. palludosa* contain a spectrum of bacteria; *Staphylococcus aureus*, *Streptococcus pneumonia*, *Staphylococcus pyrogens*, *Serratia marscessans*, *Escherichia coli*, *Streptococcus epidermidis*, *Micrococcus* and fungi: *Sacchromyces cerevisiae*, *Aspergillus spp*, *penicillium spp*. The microbial load was high in fresh samples than in cooked samples. Cooking also completely eliminated the coliforms.

The microbial load and the coliform counts were in both fresh samples, it was higher in fresh samples of *E. radiata* compared with *P. palludosa*. The high coliform counts indicate pathogenicity. However, in both *P. palludosa* and *E. radiata* cooking reduced the microbial load effectively and even eliminated the coliforms. Cooking as a processing method utilized by consumers is an effective means of preventing infections arising from the consumption of these aquatic food. The effectiveness of cooking as a means of preventing infection from these aquatic foods is best seen from the frequency of microbial and fungi isolates which were significantly reduced.

The results demonstrated that *P. palludosa* were contaminated with different types of bacterial and fungi species like *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyrogens*, *Serrata marcesseus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Micrococcus*. The fungi species were *Sacchromyces cerevisiae*, *Aspergillus spp*, *penicillium spp*. Similarly, for *E. radiata*, the contaminating species were *Bacillus spp.*, *Vibro spp.*, *Escherichia coli*, *Candida albicans*, *Streptococcus pneumonia* (*scanty*), *Sacchromyces cerevisiae* (yeast), *Serratia marscessens* (*scanty*) and *Streptococcus aureus*.

Although, the microbial load of *E. radiata* and *P. palludosa* caught from south south Nigerian tropical water has not been previously reported and compared. The studies by Frazier and Westhoff [17] have reported microbial (bacterial) infection of *P. palludosa* and *E. radiata*. Furthermore, their report indicated that *Bacillus spp.* and *Staphylococcus spp.* were the dominant type of bacteria infecting these sea foods.

Antai [18] indicated that high microbial load could in the samples is a clear indication that the fresh samples of *E. radiata* serve as a medium through which microbes multiplied rapidly. From biochemical and nutritional standpoint both *E. radiata* and *P. palludosa* are protein rich foods and therefore suitable substrates in supporting growth of different types of bacteria and fungi.

Microbial growth in these sea foods will encourage spoilage and for peasants in particular economic loss during storage. Besides, it is important that peasants who consume these sea foods are enlightened that consumption of poorly processed and cooked *E. radiata* or *P. palludosa* could predispose to health hazards such as typhoid, urinary tract infection, cholera and related infection amongst others. The presence of *Enterobacteria* in these edible mollusks is indicative of possible sewage pollution, the common contaminant in polluted littoral zones, a report which has been highlighted by Akamatsu [19].

The most noticed of the isolates is *Staphylococcus spp* present in both *E. radiata* and *P. palludosa* and are known to cause food poisoning in man. The presence of *Streptococcus spp.* indicates that *E. radiata* and *P. palludosa* may have been harvested in fresh water that has been contaminated possible with fecal matter.

The results of this study on microbial investigation also suggest

that *E. radiata* and *P. palludosa* could serve as a medium or substrate for growth of microorganisms which may be required for laboratory research and industrial processes. Hence, *E. radiata* and *P. palludosa* can be a good source of the microorganism for industrial benefits. It is important to highlight the fact that microbial growth must be controlled in order to encourage desired fermentation in industrial processes or to discourage growth of spoilage organism and pathogens in the interest of public health.

Nevertheless, factors such as the availability of water, nutrients, pH and storage temperature could determine which microorganism can grow in a particular food product and the rate at which they can grow. Bacteria tend to grow faster in fresh meal products than yeast and mold [20]. This is consistent with the present findings from the frequency data of bacteria compared with the yeast and mold (fungi).

In summary, microbial data taken together has identified a spectrum of bacteria and fungi present in *E. radiata* and *P. palludosa* food samples. The microbial load is very high in fresh than the cooked species. Cooking also completely eliminated the coliforms. *E. radiata* and *P. palludosa* have in the past caused and still gives rise to epidemics of typhoid, as a precaution therefore *E. radiata* and *P. palludosa* should be subjected to adequate processing and proper cooking before consumption in the interest of public health. However, *E. radiata* and *P. palludosa* could serve as a substrate for growing microorganisms needed for laboratory and industrial processes.

Conclusion

Fresh edible portions of *E. radiata* and *P. palludosa* have high microbial loads which are reduced by cooking, hence adequate processing and proper cooking is required of these sea foods before consumption. Also, the abundant microbial loads in these species of sea animals could serve as a ready source of microbes for use in industries.

References

1. Pascale L, Annie P, Cedric B, Bruno D (2000) Phytoplankton composition and selective feeding by the pearl oyster *pinctada margaritifera* in the takapoto lagoon (Treamotus archipelago; French Polynesia): In situ study using optical microscope and HPLC pigment analysis. Mar Ecol Prog Ser 199: 55-67.
2. Stange LA (2004) Featured creatines from the Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Services, University of Florida. (EENY-323).
3. Medale F, Lefevra F, Corraza G (2003) Qualite' nutritionnelle et dietetique des poisons constituents de la chair et facteurs de variation. Cahiers de Nutrition et de Dietetique 38: 37-44.
4. Véronique S, Marine O, Nawel B, Jean-Luc V, Jean-Charles L (2008) Lipid and fatty acid composition of fish and seafood consumed in France: CALIPSO study. J Food Compos Anal 21: 8-16.
5. Wardlaw GM, Kessel MW (2002) Perspective in Nutrition. (5thedn), Mc Graw-Hill Companies.
6. US Department of Agriculture, Agricultural Research Service (USADA-ARS), 2005. U8DA Nutrient data base for standard reference release 18.
7. Ifon ET, Umoh IB (1987) Biochemical and nutritional evaluation of *Ergeria radiata* (clam), a delicacy of some riverine peasant population in Nigeria. Food Chem 24: 21-27.
8. Ndem JJ, Akpanabiatu MI, Essien EU (2008) Effect of sea foods (periwinkle, bonefish and crayfish) and vegetable oil enriched meals on cardiovascular disease. Pak J Nutri 7: 603-606.
9. Lennette EH, Spaulding EH, Truant JP (1974) Manual of Clinical Microbiology. (2thedn) Washington DC: American Society of Microbiology Press.
10. Cheesebrough M (1987) Medical laboratory Manual for Tropical Countries. England ELBS Publishers.
11. Biomerieux API (1989) Apparatus and Products of identification (API) in Microbiology. Paris: Marcy Etiole Press.
12. Holts YG (Ed) (1982) The Shorter Bergey's Manual of Determinative Bacteriology. (8thedn) Baltimore: The Williams and Wilkins Company.
13. Hunter BB, Bennette HL (1973) Deuteromycetes (Fungi imperfect). In: Laskin AI, Lechevaliec, Cleveland Ohio: organismic Microbiology CRS Press.
14. Harrign WE, McCance ME (1976) Laboratory Methods in Food and Dairy Microbiology. London: Sterling.
15. Cowan ST, Steel EJ (1974) Identification of Medical Bacteria. (2thedn) Cambridge: Cambridge University Press.
16. Gerhardt P, Murray RGE, Erleg NR, Philips CB (1981) Manual of Methods of General Bacteriology. Washington.
17. Frazier WC, Westhoff DC (1986) Contamination, Spoilage and Preservation of Fish and other Sea Foods. Food Microbiol. McGraw-Hill Inc, New York.
18. Antai SP (1988) Study of the microbial flora of Nigeria species. Int J Food Microbiol 6: 259-261.
19. Akamatsu M (1983) Bacteriological studies on the spoilage of fish. I. Number of bacteria present in fresh fish on the market. Jap Sect Sci Fish Bul 25: 245-246.
20. Grazyna ED, Bonnie SP (2010) Environmental Effects on Seafood Availability, Safety, and Quality. Chemical & Functional Properties of Food Components. CRC Press