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 radiate (clam) were undertaking 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 fresh samples of E. Radiate (2.20 × 10⁶ - TNT CFU/ml) was significantly (p<0.05) higher compared with P. palludosa (6.10 × 10⁵ - 3.30 × 10⁴ CFU/ml). The coliform count was also significantly (p<0.05) higher in E. radiate (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 pneumonia were most frequent, a total of 20 bacteria were isolated from E. radiate, Vibro spp. and E. coil being most frequent. The three fungi isolates from fresh E. radiate 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. radiate; 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 radiate) are bivalves mollusks with two shells that provide protection to the soft body. There are over 15,000 different species of these seafoods 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 radiate from riverine areas in Nigeria is rich in protein and vital elementsand 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 seafoods are harvested from muddy and contaminated rivers. Ergeria radiate on the other hand is found in big rivers with high rate of oil spills such as Ibeno, Calabar-Itui 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 calcareous species. Reports on the microbial evaluation of E. radiate and Pomacea palludosa are however scanty.

It is therefore the aim of this study to undertake a comparative microbial evaluation of Ergeria radiate 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. radiate 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 Akamkpa 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 radiate and P. palludosa were removed from their calcareous shells, for E. radiate, 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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Reagents: The reagents were mainly BDH chemicals prepared as specified by Lennette et al. [9].

Preparation of media


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

Surface spreading technique was used to determine the total number of aerobic heterotrophic bacteria present in the sample. Serial dilution of the samples were prepared from 10^-1 to 10^-8 and 0.1 ml of each dilution was plated onto MacConkey and nutrient agar containing 10% lactic acid per ml 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^-8 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 Harrign 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 cultures 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 ± 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 Egeria radiate. Table 1 presents the total microbial count in fresh and cooked samples of Pomecia palludosa and Egeria radiate. 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 × 10^8 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 Egeria radiate ranges from 2.20 × 10^4 CFU/ml to too numerous to count and 4-10 coliforms respectively. The values for cooked Egeria radiate were 3.00 × 10^5 - 1.70 × 10^7 CFU/ml and zero coliforms. The microbial load and coliform counts were higher in the fresh E. radiate (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. radiate and Pomecia palludosa

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

The results show that Staphylococcus aureus, Streptococcus pneumonia, Streptococcus pyrogenes, Serratia marcescens, Escherichia coli, Micrococcus, Bacillus spp, Staphylococcus epidermidis, Escherichia coli, Staphylococcus aureus, and Streptococcus pneumonia, have higher frequency than other isolates. Staphylococcus aureus, Streptococcus pneumonia, St. pyrogenes and E. coli have been implicated in pathogenicity.

Table 1: Total microbial count in fresh sun dried and cooked samples of Pomecia palludosa and Egeria radiate.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range value of total count (CFU/ml)</th>
<th>Range values of no. of coliform per 100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomecia palludosa (fresh sun dried)</td>
<td>6.10 × 10^3 - 3.30 × 10^8</td>
<td>3-6 coliform/100 ml</td>
</tr>
<tr>
<td>Pomecia palludosa (cooked)</td>
<td>3.80 × 10^3 - 2.50 × 10^8</td>
<td>10-20 coliform/100 ml</td>
</tr>
<tr>
<td>Egeria radiate (fresh sun dried)</td>
<td>2.20 × 10^3 - TNT</td>
<td>4-10 coliform/100 ml</td>
</tr>
<tr>
<td>Egeria radiate (cooked)</td>
<td>3.00 × 10^5 - 1.70 × 10^3</td>
<td>0 coliform/100 ml (no growth)</td>
</tr>
</tbody>
</table>

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 Saccharomyces cerevisiae, Aspergillus spp., penicillium spp. The Saccharomyces 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. radiate were Bacillus spp., Vibro spp., Escherichia coli, Candida albicans, Streptococcus pneumonia (scanty), Sacchromyces cerevisiae (yeast), Serratia marscensess (scanty) and Streptococcus aureus. The total microbial frequencies of 26 and 20 were recorded for P. palludosa and E. radiate respectively. Staphylococcus aureus and Streptococcus pneumoniae most in P. palludosa; both organisms alongside E. coli have been implicated in pathogenesis. The microbial isolates from fresh E. radiate shows higher frequencies of Vibro spp., E. coli, Streptococcus pneumoniae, 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. radiate**

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. radiate to 4 in cooked samples of E. radiate.

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

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 cerevisia being most occurring. Aspergillus implicated in Aspergillosis, a fungi infection responsible for fungi food poisoning. However, in E. radiate, two types of yeast isolates S. cerevisiae and Candida albicans spp. were identified. No fungi isolate were identified in cooked samples of both E. radiate and P. palludosa.

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

<table>
<thead>
<tr>
<th>SN</th>
<th>Microbial isolate</th>
<th>Pomecia palludosa</th>
<th>Ergeria radiate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus spp</td>
<td>1 (33.33)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella</td>
<td>1 (33.33)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>Streptococcus spp</td>
<td>1 (33.33)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>4</td>
<td>Staphylococcus spp</td>
<td>0 (0)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>5</td>
<td>E. coli</td>
<td>0 (0)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3 (100)</td>
<td>4 (100)</td>
</tr>
</tbody>
</table>

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 4: Frequency of fungi in fresh Pomecia palludosa and Ergeria radiate.

<table>
<thead>
<tr>
<th>SN</th>
<th>Microbial isolate</th>
<th>Pomecia palludosa</th>
<th>Ergeria radiate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saccharomyces cerevisae (yeast)</td>
<td>10 (57)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>2</td>
<td>Aspergillus spp</td>
<td>8 (27)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>Penicillium spp</td>
<td>9 (33)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>Candida spp (yeast)</td>
<td>0 (0)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27 (100)</td>
<td>8 (100)</td>
</tr>
</tbody>
</table>

Values are presented as frequency and percentages (in parenthesis)

-Aspergillus is implicated in Aspergillosis a fungal infection through food
- Candida albicans has been implicated in candidiasis common in women.

### Discussion

The 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 marscensess, 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. radiate compared with P. palludosa. The high coliform counts indicate pathogenicity. However, in both P. palludosa and E. radiate 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, Serratia marscensess, Escherichia coli, Staphylococcus epidermidis and Micrococcus. The fungi species were Saccharomyces cerevisiae, Aspergillus spp, penicillium spp. Similarly, for E. radiate, the contaminating species were Bacillus spp., Vibro spp., Escherichia coli, Candida albicans, Streptococcus pneumonia (scanty), Sacchromyces cerevisiae (yeast), Serratia marscensess (scanty) and Streptococcus aureus.

Although, the microbial load of E. radiate 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. radiate. 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. radiate serve as a medium through which microbes multiplied rapidly. From biochemical and nutritional standpoint both E. radiate 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. radiate 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. radiate and P. palludosa and are known to cause food poisoning in man. The presence of Streptococcus spp. indicates that E. radiate 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* could 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. radiate* 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**


