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Full Length Research Paper

Comparative microbial evaluation of two edible seafood*P. palludosa* (apple snail) and *E. radiata* (clam) to ascertain their consumption safety

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Microbial evaluation of two edible seafood, *Pomecia palludosa* (apple snail) and *Ergeriaradiate* (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 thatthe bacteria loadin fresh samples of *E. Radiate*(2.20 x 10^6 - TNT CFU/ml) was significantly (p<0.05) higher compared with *P. palludosa*(6.10 x 10^3 - 3.30 x 10^8 CFU/ml). The coliform count was also significantly (p<0.05) higher in *E. Radiate*(4-10 coliform/100ml) than in *P. palludosa*(3-6 coliform/100ml). 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. radiata, Vibro spp.* and *E. coli*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.

Key words: *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 radiate*) 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 (Pascale et al., 2000; Stange, 2004).

Thesesea-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, (Medele et al., 2003).

The omega-3 fatty acids are involved in the prevention of cardiovascular diseases (Véronique et al., 2008). 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 (Wardlaw and Kessel, 2002; USDD-ARS, 2005).

Report by Ifon and Umoh (1987) also indicates that *Ergeriaradiata* from riverine areas in Nigeria is rich in protein and vital elementsand their protein content compares reasonably well with values obtainedfrom whole hens' eggs, this further justifies the consumption of these seafood's as cheap and good sources of animal proteins. (Ndem et al., 2008; Véronique et al., 2008). Nevertheless, this sea food's are harvested from muddy and contaminated rivers. *Ergeriaradiata* on the other hand is found in big rivers with high rate of oil spills such as Ibeno, Calabar-Itu rivers etc, whereas *Pomeciapalludosa* 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 *Pomeciapalludosa* are however scanty.

It is therefore the aim of this study to undertake a comparative microbial evaluation of *Ergeria radiate* and *Pomecia palludosa* in order to ascertain their safety for

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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 *Pomecia 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. *Ergeriaradiata*samples were freshly harvested from Calabar Itu bridge beach market in AkwaIbom 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 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^{o}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.

Reagents: The reagents were mainly BDH chemicals prepared as specified by Lennette*et al*., (1974).

Preparation of media

The recipe for preparation of media used followed the methods described by Cheesberough (1987) in Biomerieux API, 1989.

Enumeration of aerobic heterotropic 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^{-10} and 0.1ml 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^{o}C for 24 hours before enumeration.

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. 0.1ml 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^{o}C for 72 hours before enumeration.

Viable count method (Harrign and McCance, 1976)

All plating and counts were done by the pour plate technique of Harrign and McCance (1976).

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^{o}C for 24hours and 28^{o}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^{o}C until needed.

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 (1981). Identification of the bacteria to the generic levels followed the scheme of Holt (1982). The fungi isolates were characterized based on the macroscopic and microscopic appearances. Their probable identities were determined according to Hunter and Bennette (1973) 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 *Ergeriaradiata*

Table 1 presents the total microbial count in fresh and cooked samples of *Pomecia palludosa* and *Ergeria radiate*.

The total microbial count for fresh sun dried *Pomecia palludosa* ranged from 6.10×10^3 to 3.30×10^8CFU/ml, their coliform counts ranged from 3 to 6 coliform/100ml respectively, values for cooked were 3.80×10^3 - 2.50×10^5CFU/ml and 10-20 coliform/100ml 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 *Ergeriaradiata* ranges from 2.20×10^6 CFU/ml to too numerous to count and 4-10 coliforms respectively. The values for cooked *Ergeriaradiata* were 3.00×10^3 - 1.70×10^5CFU/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. radiate* 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, Serratamarcesseus, Escherichia coli, Staphylococcus epidermidis*and *Micrococcus* were bacteria identified in *P. palludosa*. The corresponding fungi were *Sacchromyluscerevisae, Aspergillus spp, penicillium spp*. The *Sacchromycescerevisae* was found in all samples while samples 1, 2, 5, 7 and 8 contain *Penicillum* 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, Streptococcuspneumonia*(scanty), *Sacchromycescerevisae (yeast), Serratiamarscessens (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 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. cerevisae (yeast), Aspergillus spp.* and *Penicillum spp*.) with *Saccharomyces cereviva* being most occurring. *Aspergillus*is implicated in *Aspergillosis*, a fungi infection responsible for fungi food poisoning. However, in *E. radiata*, two types of yeast isolates *S. Cerevisae* and *Candida albicans spp.* were identified. No fungi isolate were identified in cooked samples of both *E. radiate* and *P. palludosa*. Table 4.

DISCUSSION

This 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 aureaus, Streptococcus pneumonia, Staphylococcus pyrogens, Serratiamarscessans, Escherichia coli, Streptococcus epidermidis, Micrococcus* and fungi: *Sacchromycescerevisae, Aspergillus spp, penicillum 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. radiata* cooking reduced the microbial load effectively and even eliminated the coliforms. Cooking as a processing method utilized by consumers

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

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

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

SN	Microbial isolate	*Pomecia palludosa*	*Ergeria Radiate*
1	***Staphylococcus aureus***	7 (26)	3 (17)
2	***Streptococcus pneumonia***	6 (23)	1 (6)
3	***Streptococcus pyrogens***	4 (15)	0 (0)
4	***Serratiamarcessens***	3 (12)	2 (11)
5	***Micrococcus***	1 (4)	0 (0)
6	***Escherichia coli***	3 (12)	4 (22)
7	***Staphylococcus epidermidis***	2 (8)	0 (0)
8	***Bacillus spp.***	0 (0)	2 (11)
9	***Vibrospp.***	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.

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 food is best seen from the frequency of microbial and fungi isolates which were significantly reduced.

The results demonstrated that *P. Palludosa* was contaminated with different types of bacteria and fungi species like *Staphylococcus aureus, Streptococcus pneumonia,Streptococcuspyrogens,Serratamarcessens , Escherichia coli, Staphylococcus epidermidis* and *Micrococcus.* The fungi species were *Sacchromyluscerevisae, Aspergillus spp, penicillium spp*. Similarly, for *E. radiata*, the contaminating species were *Bacillus spp., Vibro spp., Escherichia coli, Candida albicans, Streptococcus pneumonia (*scanty*), Sacchromycescerevisa*e (yeast), *Serratiamarscessens (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 Westh off (1986) 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 seafood.

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

SN	Microbial isolate	*Pomecia palludosa*	*Ergeria radiata*
1	*Bacillus spp*	1 (33.33)	1 (25)
2	*Salmonella*	1 (33.33)	0 (0)
3	*Streptococcus spp*	1 (33.33)	1 (25)
4	*Staphylococcus spp*	0 (0)	1 (25)
5	*E. coli*	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 4. Frequency of fungi in fresh *Pomecia palludosa* and *Ergeria radiate.*

SN	Microbial isolate	Pomecia palludosa	Ergeria Radiate
1	Saccharomyces cereviva(yeast)	10 (37)	4 (50)
2	Aspergillusspp.	8 (27)	0 (0)
3	Penicillin spp.	9 (33)	0 (0)
4	Candida spp. (yeast)	0 (0)	4 (50)
Total		27 (100)	8 (100)

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

Antai (1998) 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. Radiate* and *P. palludosa* are protein rich food and therefore suitable substrates in supporting growth of different types of bacteria and fungi.

Microbial growth in these sea food will encourage spoilage and for peasants in particular economic loss during storage. Besides, it is important that peasants who consume these sea food are enlightenedthat 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 (1983).

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. radiate* and *P. palludosa* could serve as a medium or substrate for growth of microorganisms which may be required for laboratory research and Hence, *E. radiate* 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 (Grazyna and Bonnie, 2010). 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. radiate* and *P. palludosa* food samples. The microbial load is very high in fresh than the cooked species. Cooking also completely eliminated the coliforms. *E. radiate* and *P. palludosa* have in the past caused and still gives rise to epidemics of typhoid, as a precaution therefore*E. Radiate* and *P. Palludosa*should be subjected to adequate processing and proper cooking before consumption in the interest of public health. However, *E. radiate* and *P. palludosa* could serve as substrates 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 food 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.

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