# EFFECT OF *Citrus aurantifolia* AND SODIUM CHLORIDE ON BACTERIOLOGICAL AND NUTRITIONAL QUALITY OF FRESH *Tympanotonous fuscatus*

Supported by tetfund

**M. U. Okon*, C. U. Inyang and O. J. Akinjogunla**
Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria
*Corresponding author: matthewumanah@gmail.com

**Received:** March 19, 2020 **Accepted:** November 13, 2020

Abstract: Effect of single and combination of *Citrus aurantifolia* and sodium chloride (NaCl) on the bacterial loads and proximate composition of fresh *Tympanotonous fuscatus* were determined using standard bacteriological and analytical techniques. The results showed 99.97% reduction in total heterotrophic bacterial counts on *T. fuscatus* treated with ≤ 10% NaCl and *C. aurantifolia* for 5 min. The results showed 99.9% reduction in total heterotrophic bacterial, total coliform and total faecal counts of *T. fuscatus* treated with 10% NaCl and *C. aurantifolia* for ≤ 10 min. The predominant bacterial genera in the treated *T. fuscatus* were *Bacillus*, *Staphylococcus*, *Acinetobacter* and *Micrococcus*. There was no significant difference ($p \geq 0.05$) on the moisture, ash, fiber content, crude lipids, crude protein and total carbohydrate content of both treated and untreated *T. fuscatus*. This study showed that *T. fuscatus* sample could be treated with 10% *C. aurantifolia* and NaCl alone or in combination for 10 min to prevent possible foodborne diseases associated with this aquatic food.

**Keywords:** Bacteriological, *Citrus aurantifolia*, NaCl, *Tympanotonous fuscatus*

## Introduction

Shellfish and fish constitute high nutritional values and special delicacies to humans worldwide and also provide a great source of wealth to riverine territorial dwellers like the Niger Delta region of Nigeria (Wafaa *et al*., 2011). Shellfishes such as periwinkles harboured pathogenic microorganisms that caused food-borne diseases in many parts of the world (Jatua *et al*., 2014). Most aquatic foods contain diverse amount of saturated, monounsaturated and polyunsaturated fat with shellfish containing 15% fat calories. Shellfish also provide a high quality protein with all the dietary essential amino acids for maintenance and growth of the human body (FNB, 2007).

*Tympanotonous fuscatus* (periwinkles) are invertebrates, belonging to the phylum "*Mollusca"*, class "*Gastropoda"* and subclass "*Prosobranchia",* commonly found in the littoral region of sea, brackish or estuaries waters (Dorit *et al*., 1991). In Nigeria, periwinkles are found in the Lagoons, mangrove swamps and are represented by two genera: *Tympanotonous and Pachymelania* (Adebayo-tayo *et al*., 2006; Oyenekan, 2010). *Tympanotonous fuscatus* has medicinal properties for treatment of endemic goiter due to its iodine content (Ogungbenle and Omowole, 2012); contains essential minerals such as calcium, magnesium, zinc, copper, manganese, sodium, potassium and iron (Ogungbenle and Omowole, 2012) and are also consumed in mass as a cheap source of animal protein (Ekanem and Otti, 1997). Besides the nutritional values of *T. fuscatus*, it is used in treating pimples, cleansing and as well as calcium source for animal when the shell is grounded (Bob-mauel, 2012). The *T. fuscatus* harboured microorganisms such as *Salmonella paratyphi*, *Enterobacter aerogenes*, *Micrococcus varians*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* (Adebayo-tayo *et al*., 2006).

Sodium chloride (common salt) is one of the most commonly used additives as well as preservatives in food industries because of its low cost and varied properties. It has a preservative and antimicrobial effect as a direct consequence of its capacity to reduce water activity values for pathogenic or spoilage organisms (Man, 2007). Sodium chloride (NaCl) also enhances flavour and the flavouring enchancing effects differ from reducing or enchancing the enzymatic activities of some enzymes responsible for the development of different organoleptic parameters (Cheng *et al.*, 2003). Several researchers have suggested the use of natural antimicrobial compounds for food preservative owing to the hazardous effects of some chemical preservatives can pose to human health. Among the natural products, *Citrus aurantifolia* (lime juice) have been approved and given the Generally Recognized as Safe (GRAS) status for its application in food (Skrivanova *et al*., 2011). The antimicrobial activities of *C. aurantifolia*, an organic acid fruit, have been reported (Riche, 2003; Davidson and Taylor, 2007). However, the use of NaCl as a sole preservative is totally ineffective in some food products, justifying its combination with other preservatives for efficient results. The aim of this study was to determine the effect of *C. aurantifolia* and sodium chloride on the bacteriological and nutritional quality of fresh *T. fuscatus.*

## Materials and Methods

### *Collection of samples*

Twenty (20) *Tympanotonus fuscatus* (periwinkle) were obtained from two major markets in Uyo (Itam and Akpan Andem markets) using sterile wide-mouth containers and were immediately transported to the Department of Microbiology Laboratory, University of Uyo. The *T. fuscatus* was identified and confirmed by a Fish Taxonomist in the Department of Fisheries and Aquaculture, University of Uyo. The samples were extensively washed with sterile distilled water and rinsed with normal saline to remove all extraneous materials before shucking; thereafter the edible part was aseptically removed and transferred into sterile containers for bacteriological analysis.

### *Bacteriological analysis of samples*

Ten (10) grams of each fleshy blended parts of the *T. fuscatus* sample was suspended into 90 ml peptone water and vigorously shaken to dislodge adhered bacteria. Ten-fold serial dilutions were made to obtain dilution of $10^{-1}$ and $10^{-3}$, One (1) ml of aliquot was pour-plated in triplicate onto each plate of Nutrient Agar (NA), MacConkey Agar (MCA), Eosine Methylene Blue (EMB) Agar and the plates were incubated at 37°C overnight. After incubation, colonies on plates were counted and multiplied by the dilution to obtain the Total Heterotrophic Bacterial Counts (THBC), Total Coliform Counts (TCC) and Total Faecal Coliform Counts (TFC), respectively. The discrete colonies were sub-cultured onto freshly prepared nutrient agar plates and aerobically incubated overnight at 37°C. The pure cultures of isolates were streaked onto nutrient agar slant, incubated at 37°C and stored in a refrigerator at 4°C for characterization and identification. All isolates were Gram stained and subjected to convectional biochemical tests (Holt *et al*., 1994).

***Effect of Citrus aurantifolia (lime juice) and sodium chloride (NaCl) on the bacterial loads of T. fuscatus***

Fleshy part of the *T. fuscatus* sample was suspended into sterile conical flasks containing varied concentrations (2.5, 5.0, 7.5 and 10%) of NaCl and *C. aurantifolia*, respectively. The contents of sterile conical flasks were allowed to stand for 5 and 10 min, respectively. Thereafter, 10 g of each fleshy part was blended, separately suspended into 90 ml peptone water and vigorously shaken to dislodge adhered bacteria. Serial dilutions were made to obtain dilutions of $10^{-1}$ and $10^{-3}$. One (1) ml of the aliquot was pour-plated in triplicate onto each plate of Nutrient Agar (NA), MacConkey Agar (MCA), Eosine Methylene Blue Agar (EMB), and incubated aerobically at 37°C for 24 h. The same procedure was carried out for mixture of NaCl and *C. aurantifolia* at ratio of 1:1 (vol/vol). The same procedures were carried on *T. fuscatus* untreated with NaCl and *C. aurantifolia* (served as the control). After incubation, the bacterial counts were recorded, mean and standard deviation calculated.

***Proximate analysis of T. fuscatus treated with sodium chloride and Citrus aurantifolia***

The moisture and ash contents of the samples were determined using gravimetric methods (AOAC, 2005). The lipid content was determined using Soxhlet extract method (AOAC, 2005). The crude protein and fibre contents were done using Kjeldahl's procedure and subsequent conversion to crude protein by multiplying the values obtained with a protein conversion factor of 6.25 (AOAC, 2005). The energy content was calculated as follows: Energy Kcal 100 g = (crude lipid x 8) + (crude protein x 2) + (CHO x 4), Where CHO is carbohydrate contents of the *T. fuscatus*. All determinations were in triplicates and the same was repeated for all samples and values obtained were expressed as mean ± standard deviation (mean ± SD).

**Results and Discussion**

The logarithmic and percentage reductions of bacterial loads in *T. fuscatus* treated with NaCl are presented in Table 1. The results showed a reduction in THBC of *T. fuscatus* sample treated with 10% NaCl for 5 min from $5.8 \pm 0.1 \times 10^4$ to $1.41 \pm 0.8 \times 10^2$ CFU/g (99.75% reduction), while *T. fuscatus* treated with 10% NaCl for 10 min reduced in THBC from $5.8 \pm 0.1 \times 10^4$ to $1.5 \pm 0.8 \times 10^1$ CFU/g (99.97% reduction).

**Table 1: Logarithmic and percentage reductions of bacterial isolates in *Tympanotonus fuscatus* treated with sodium chloride**

| Exposure Time (min) | M.G. | Conc. (%) | Plate Counts (CFU/g) | Log (CFU/g) | % Redu. | Log Redu. |
|---|---|---|---|---|---|---|
| 5 | THBC | 0 | $5.8 \pm 0.1 \times 10^4$ | 4.763 | NA | NA |
| | | 2.5 | $5.1 \pm 0.3 \times 10^4$ | 4.708 | 12.06 | 0.06 |
| | | 5.0 | $3.5 \pm 0.7 \times 10^3$ | 3.544 | 93.96 | 1.22 |
| | | 7.5 | $2.3 \pm 0.3 \times 10^2$ | 2.362 | 99.60 | 2.40 |
| | | 10 | $1.4 \pm 0.8 \times 10^2$ | 2.146 | 99.75 | 2.62 |
| | TCC | 0 | $4.0 \pm 0.4 \times 10^3$ | 3.602 | NA | NA |
| | | 2.5 | $2.7 \pm 0.3 \times 10^3$ | 3.431 | 32.50 | 0.17 |
| | | 5.0 | $1.4 \pm 0.8 \times 10^2$ | 2.146 | 62.00 | 1.46 |
| | | 7.5 | $1.1 \pm 0.3 \times 10^2$ | 2.041 | 97.25 | 1.56 |
| | | 10 | NG | NA | ≥ 99.99 | 3.60 |
| | TFC | 0 | $2.0 \pm 0.7 \times 10^2$ | 2.301 | NA | NA |
| | | 2.5 | $1.2 \pm 0.3 \times 10^2$ | 2.079 | 40.00 | 0.22 |
| | | 5.0 | NG | NA | ≥ 99.99 | 2.30 |
| | | 7.5 | NG | NA | ≥ 99.99 | 2.30 |
| | | 10 | NG | NA | ≥ 99.99 | 2.30 |
| 10 | THBC | 0 | $5.8 \pm 0.1 \times 10^4$ | 4.763 | NA | NA |
| | | 2.5 | $4.5 \pm 0.7 \times 10^4$ | 4.653 | 22.41 | 0.11 |
| | | 5.0 | $2.8 \pm 0.1 \times 10^3$ | 3.447 | 95.17 | 1.32 |
| | | 7.5 | $1.9 \pm 1.4 \times 10^2$ | 2.279 | 99.67 | 2.48 |
| | | 10 | $1.5 \pm 0.8 \times 10^1$ | 1.176 | 99.97 | 3.59 |
| | TCC | 0 | $4.0 \pm 0.4 \times 10^3$ | 3.602 | NA | NA |
| | | 2.5 | $1.8 \pm 0.1 \times 10^2$ | 2.255 | 95.50 | 1.35 |
| | | 5.0 | $1.0 \pm 0.0 \times 10^2$ | 2.000 | 97.50 | 1.60 |
| | | 7.5 | NG | NA | ≥ 99.99 | 3.60 |
| | | 10 | NG | NA | ≥ 99.99 | 3.60 |
| | TFC | 0 | $2.0 \pm 0.7 \times 10^2$ | 2.301 | NA | NA |
| | | 2.5 | NG | NA | ≥ 99.99 | 2.30 |
| | | 5.0 | NG | NA | ≥ 99.99 | 2.30 |
| | | 7.5 | NG | NA | ≥ 99.99 | 2.30 |
| | | 10 | NG | NA | ≥ 99.99 | 2.30 |

M.G.: Microbial Group; THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Coliform Counts; NG: No Growth; NA: Not Applicable; CFU: Colony Forming Units; Log: Logarithmic

**Table 2: Logarithmic and percentage reductions of bacterial isolates in *Tympanotonus fuscatus* treated with *Citrus aurantifolia***

| Exposure Time (min) | M.G. | Conc. (%) | Plate Counts (CFU/g) | Log (CFU/g) | % Redu. | Log Redu. |
|---|---|---|---|---|---|---|
| 5 | THBC | 0 | $5.8 \pm 0.1 \times 10^4$ | 4.763 | NA | NA |
| | | 2.5 | $2.4 \pm 0.5 \times 10^4$ | 4.380 | 58.62 | 0.38 |
| | | 5.0 | $2.0 \pm 0.7 \times 10^3$ | 3.301 | 96.55 | 1.46 |
| | | 7.5 | $1.6 \pm 0.2 \times 10^2$ | 2.204 | 99.72 | 2.56 |
| | | 10 | $1.0 \pm 0.4 \times 10^2$ | 2.000 | 99.83 | 2.76 |
| | TCC | 0 | $4.0 \pm 0.4 \times 10^3$ | 3.602 | NA | NA |
| | | 2.5 | $1.2 \pm 0.3 \times 10^3$ | 3.079 | 70.00 | 0.52 |
| | | 5.0 | $1.4 \pm 0.8 \times 10^2$ | 2.000 | 97.50 | 1.60 |
| | | 7.5 | NG | NA | ≥ 99.99 | 3.60 |
| | | 10 | NG | NA | ≥ 99.99 | 3.60 |
| | TFC | 0 | $2.0 \pm 0.7 \times 10^2$ | 2.301 | NA | NA |
| | | 2.5 | $1.0 \pm 0.4 \times 10^2$ | 2.000 | 50.00 | 0.30 |
| | | 5.0 | NG | NA | ≥ 99.99 | 2.30 |
| | | 7.5 | NG | NA | ≥ 99.99 | 2.30 |
| | | 10 | NG | NA | ≥ 99.99 | 2.30 |
| 10 | THBC | 0 | $5.8 \pm 0.1 \times 10^4$ | 4.763 | NA | NA |
| | | 2.5 | $1.0 \pm 0.4 \times 10^3$ | 3.000 | 37.02 | 1.76 |
| | | 5.0 | $1.3 \pm 0.4 \times 10^2$ | 2.114 | 55.62 | 2.65 |
| | | 7.5 | $1.8 \pm 0.2 \times 10^1$ | 1.255 | 73.65 | 3.51 |
| | | 10 | $1.0 \pm 0.4 \times 10^1$ | 1.000 | 79.00 | 3.76 |
| | TCC | 0 | $4.0 \pm 0.4 \times 10^3$ | 3.602 | NA | NA |
| | | 2.5 | $1.2 \pm 0.3 \times 10^2$ | 2.079 | 42.28 | 1.16 |
| | | 5.0 | NG | NA | ≥ 99.99 | 3.60 |
| | | 7.5 | NG | NA | ≥ 99.99 | 3.60 |
| | | 10 | NG | NA | ≥ 99.99 | 3.60 |
| | TFC | 0 | $2.0 \pm 0.7 \times 10^2$ | 2.301 | NA | NA |
| | | 2.5 | $1.4 \pm 0.8 \times 10^2$ | 1.146 | 50.20 | 1.16 |
| | | 5.0 | NG | NA | ≥ 99.99 | 2.30 |
| | | 7.5 | NG | NA | ≥ 99.99 | 2.30 |
| | | 10 | NG | NA | ≥ 99.99 | 2.30 |

M.G.: Microbial Group; THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Coliform Counts; NG: No Growth; NA: Not Applicable; CFU: Colony Forming Units; Log: Logarithmic

The *T. fuscatus* treated with 7.5 NaCl for 5 min reduced in TCC from $4.0 \pm 0.4 \times 10^3$ to $1.1 \pm 0.3 \times 10^2$ CFU/g (97.25% reduction), while those treated with 7.5 % NaCl at 10 min had no TCC. The TFC in *T. fuscatus* treated with 5 and 10 for ≤ 10 min reduced by 99.99 . The logarithmic and percentage reductions of bacterial loads in *T. fuscatus* treated with *C. aurantifolia* are presented in Table 2. The results showed a reduction in THBC of *T. fuscatus* treated with 10 % *C. aurantifolia* for 5 min from $5.8 \pm 0.1 \times 10^4$ to $1.0 \pm 0.4 \times 10^2$ CFU/g 2.76 log reduction), while *T. fuscatus* treated with 10% *C. aurantifolia* for 10 min had THBC reduction from $5.8 \pm 0.1 \times 10^4$ to $1.0 \pm 0.4 \times 10^1$ CFU/g (3.76 log reduction). The *T. fuscatus* treated with 7.5% of *C. aurantifolia* had no TCC, while *T. fuscatus* treated with 5% *C. aurantifolia* for 5 min had no TFC.

The logarithmic and percentage reductions of bacterial loads in *T. fuscatus* treated with both NaCl and *C. aurantifolia* are shown in Table 3. The THBC of *T. fuscatus* sample treated with equal concentrations (10 %) of NaCl and *C. aurantifolia* was reduced by ≥ 99.99 % within 10 min of exposure, while the TCC and TFC of *T. fuscatus* treated with equal concentrations of 5 % (NaCl and *C. aurantifolia*) within ≤ 10 min of exposure had 99.99 % reduction. The trends of occurrence of bacteria isolates from *T. fuscatus* treated with NaCl and *C. aurantifolia* singly and in combination are shown in Table 4. The results obtained showed that *B. subtilis*, *S. aureus*, *M. varians*, *C. diversus*, *V. cholerae* and *Acinetobacter* spp survived in *T. fuscatus* treated with NaCl and *C. aurantifolia* at 5% within 5 min of exposure. The *T. fuscatus* treated with NaCl and *C. aurantifolia* at 5% within 10 min of exposure had *B. subtilis*, *S. aureus*, *M. varians*, *V. cholerae* and *Acinetobacter* spp. The *T. fuscatus* treated with equal volume (5%) of NaCl and *C. aurantifolia* and left for 10 min had *B. subtilis*, *S. aureus*, *M. varians.* The results also revealed that *B. subtilis* and *M. varians* were predominant in all the treated samples, except when NaCl and *C. aurantifolia* were combined at 10% concentration for 10 min.

**Table 3: Logarithmic and percentage reductions of bacterial isolates in *Tympanotonus fuscatus* treated with *Citrus aurantifolia* and sodium chloride**

| Exposure Time (min) | M.G. | Conc. (%) | Plate Counts (CFU/g) | Log (CFU/g) | % Redu. | Log Redu. |
|---|---|---|---|---|---|---|
| | | 0 | $5.8 \pm 0.1 \times 10^4$ | 4.763 | NA | NA |
| | | 2.5 | $2.9 \pm 0.7 \times 10^4$ | 4.462 | 6.320 | 0.30 |
| | THBC | 5.0 | $1.6 \pm 0.8 \times 10^2$ | 2.204 | 53.73 | 2.56 |
| | | 7.5 | $1.4 \pm 0.4 \times 10^1$ | 1.146 | 75.94 | 3.62 |
| | | 10 | NG | NA | ≥ 99.99 | 4.76 |
| | | 0 | $4.0 \pm 0.4 \times 10^3$ | 3.602 | NA | NA |
| | | 2.5 | $1.2 \pm 0.3 \times 10^3$ | 2.079 | 42.28 | 1.52 |
| 5 | TCC | 5.0 | NG | NA | ≥ 99.99 | 3.60 |
| | | 7.5 | NG | NA | ≥ 99.99 | 3.60 |
| | | 10 | NG | NA | ≥ 99.99 | 3.60 |
| | | 0 | $2.0 \pm 0.4 \times 10^2$ | 2.301 | NA | NA |
| | | 2.5 | NG | NA | ≥ 99.99 | 2.30 |
| | TFC | 5.0 | NG | NA | ≥ 99.99 | 2.30 |
| | | 7.5 | NG | NA | ≥ 99.99 | 2.30 |
| | | 10 | NG | NA | ≥ 99.99 | 2.30 |
| | | 0 | $5.8 \pm 0.1 \times 10^4$ | 4.763 | NA | NA |
| | | 2.5 | $1.8 \pm 0.8 \times 10^2$ | 2.255 | 52.66 | 2.21 |
| | THBC | 5.0 | $1.0 \pm 0.0 \times 10^2$ | 2.000 | 58.01 | 2.76 |
| | | 7.5 | NG | NA | ≥ 99.99 | 4.76 |
| | | 10 | NG | NA | ≥ 99.99 | 4.76 |
| | | 0 | $4.0 \pm 0.4 \times 10^3$ | 3.602 | NA | NA |
| | | 2.5 | NG | NA | ≥ 99.99 | 3.60 |
| 10 | TCC | 5.0 | NG | NA | ≥ 99.99 | 3.60 |
| | | 7.5 | NG | NA | ≥ 99.99 | 3.60 |
| | | 10 | NG | NA | ≥ 99.99 | 3.60 |
| | | 0 | $2.0 \pm 0.4 \times 10^2$ | 2.301 | NA | NA |
| | | 2.5 | NG | NA | ≥ 99.99 | 2.30 |
| | TFC | 5.0 | NG | NA | ≥ 99.99 | 2.30 |
| | | 7.5 | NG | NA | ≥ 99.99 | 2.30 |
| | | 10 | NG | NA | ≥ 99.99 | 2.30 |

M.G.: Microbial Group; THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Coliform Counts; NG: No Growth; NA: Not Applicable; CFU: Colony Forming Units; Log: Logarithmic

**Table 4: Occurrence of bacterial isolates in *T. fuscatus* treated with sodium chloride and *C. aurantifolia***

| Bacterial Isolates | A (5 min) | | | A (10 min) | | B (5 min) | | B (10 mins) | | A + B (5 min) | | A + B (10 min) | | Total No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% | |
| *B. subtilis* | + | + | + | + | + | + | + | + | + | + | + | + | - | 11 |
| *S. aureus* | + | + | - | + | - | + | + | + | - | + | - | + | - | 7 |
| *M. varians* | + | + | + | + | + | + | + | + | - | + | - | + | - | 9 |
| *Aeromonas* spp | + | + | - | - | - | + | - | + | - | - | - | - | - | 3 |
| *S. enterica* | + | - | - | - | - | + | - | + | - | - | - | - | - | 2 |
| *S. sonnei* | + | - | - | - | - | + | - | + | - | - | - | - | - | 2 |
| *S. pyogenes* | + | - | - | - | - | + | + | + | - | - | - | - | - | 3 |
| *C. diversus* | + | + | - | + | - | + | + | - | - | - | - | - | - | 4 |
| *K. pneumoniae* | + | + | - | + | - | - | - | - | - | - | - | - | - | 2 |
| *E. faecium* | + | - | - | - | - | + | + | + | - | - | - | - | - | 3 |
| *V. cholerae* | + | + | - | + | - | + | + | + | - | + | - | - | - | 6 |
| *E. coli* | + | - | - | - | - | + | - | + | - | - | - | - | - | 2 |
| *Acinetobacter* spp | + | + | - | + | - | + | + | + | - | + | - | + | - | 7 |
| *P. mirabilis* | + | - | - | - | - | + | - | + | - | - | - | - | - | 2 |
| Total | 14 | 8 | 2 | 7 | 2 | 13 | 8 | 12 | 1 | 5 | 1 | 4 | 0 | 63 |

A: Sodium Chloride; B: *Citrus aurantifolia*; A + B: Sodium Chloride + *Citrus aurantifolia*; +: Present; – : Not Present

**Table 5: Comparative analysis of proximate parameters of *T. fuscatus* treated with sodium chloride and *Citrus aurantifolia***

| Sample | Treatment | Percentage / mm ± S.D | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Moisture | Ash | Fiber Content | Crude Lipid | Crude Protein | Total CHO | Calorie Value (kcal) |
| $P_a$ | NaCl | 80 ± 1.00 | 3.859±0.03 | 0.920±0.02 | 6.320±0.01 | 41.220±0.25 | 47.677±0.00 | 412.468±0.004 |
| $P_b$ | *C. aurantifolia* | 80 ± 1.00 | 3.859±0.01 | 0.922±0.01 | 6.322±0.02 | 41.222±0.22 | 47.675±0.12 | 412.486±0.001 |
| $P_c$ | NaCl and *C. aurantifolia* | 72 ± 2.00 | 3.849±0.02 | 0.508±0.02 | 6.296±0.02 | 40.631±0.00 | 48.716±0.13 | 414.052±0.002 |
| $P_d$ | Control | 84 ± 2.00 | 3.860±0.01 | 0.932±0.02 | 6.320±0.02 | 41.229±0.10 | 47.659±0.15 | 412.432±0.001 |

Each value represents the means of triplicate plate counts and standard deviation; mm: mean; Standard Deviation

The effects of NaCl and *C. aurantifolia* singly and in combination on the proximate composition of *T. fuscatus* are presented in Table 5. The sample $P_d$ (control) had the highest moisture content of 84 ± 2%, followed by sample $P_a$ (*T. fuscatus* treated with 10% NaCl) and sample $P_b$ (*T. fuscatus* treated with 10% *C. aurantifolia*) with each having 80 ± 1.00%, while sample $P_c$ (*T. fuscatus* treated with both 10% NaCl and 10% *C. aurantifolia*) had the lowest moisture content of 72 ± 2.00%. The ash content was highest in sample $P_d$ (3.860 ± 0.01%), followed by samples $P_a$ and $P_b$ with each having 3.859 ± 0.01 %. The fibre content was lowest in sample $P_c$ (0.508 ± 0.02%), while sample $P_d$ had the highest fibre content (0.932 ± 0.02%). The crude protein was lowest in sample $P_c$ (40.631 ± 0.00%), while sample $P_d$ had the highest protein (41.229 ± 0.10%). The crude lipid was highest in sample $P_b$ (6.320 ± 0.02%) and was lowest in sample $P_c$ with 6.296 ± 0.02%. The total carbohydrate was highest in sample $P_c$ (48.716 ± 0.30%) and was lowest in sample $P_d$ (47.659 ± 0.15%).

Although *T. fuscatus* is an inexpensive source of protein and other nutrients, it has a tendency of harbouring microorganisms especially those that are pathogenic to humans due to the poor sanitary conditions of the water bodies where *T. fuscatus* is obtained (Adebayo-Tayo *et al*., 2006). In this study, we obtained a high THBC, TCC and FCC in *T. fuscatus* and this corroborated the reports of Ekanem and Adegoke (1995); Oranusi *et al*. (2018) who observed unacceptable levels of pathogenic and non-pathogenic bacteria in shellfishes. The significantly high bacterial loads could be attributed to poor handling and processing in the market processed fishes (Odu *et al*., 2010; Akinjogunla *et al*., 2011).

In this study, as the NaCl concentrations increased from 5 to 10%, the bacterial load of *T. fuscatus* decreased. This result is in accordance with Soyiri *et al*. (2008); Onyeagba and Isu (2006) and Anbalagan *et al*. (2014) who reported that salt concentrations between 7.5 and 10% eliminated all pathogenic bacteria from shell fishes. The increase in NaCl concentrations creates unfavourable conditions for organisms to thrive and proliferate for NaCl acts as water binding agent by removing water from the food products such as *T. fuscatus* by osmosis, thereby plasmolyzing the cell walls of pathogenic organisms (Orjimelukwe *et al.*, 2017). Findings of this study showed that the bacterial loads of *T. fuscatus* treated with varied concentrations of *C. aurantifolia* reduced to acceptable levels for human consumption and this is in conformity with the reports of Mata *et al*. (1994) and Rodrigues *et al*. (2000).

In this study, the fourteen bacterial genera obtained from the untreated *T. fuscatus* were *Staphylococcus*, *Bacillus*, *Proteus*, *Micrococcus*, *Salmonella*, *Shigella*, *Streptococcus*, *Vibrio*, *Citrobacter*, *Klebsiella*, *Enterococcus*, *Acinetobacter*, *Aeromonas* and *Escherichia.* The isolation of *E. coli*, *Proteus* spp., *M. varians* and *P. aeruginosa* from *T. fuscatus* in this study agreed with Adebayo-tayo *et al.* (2006) who obtained these bacterial isolates from *T. fuscatus* in two different creeks in Nigeria.

The moisture contents of both the control (untreated *T. fuscatus*) and *T. fuscatus* treated with *C. aurantifolia* and NaCl in our study agreed with the report of Pessu *et al*. (2014) who worked on the effects of different processing methods on the meat of *T. fuscatus*. Our findings showed no significant difference ($p \geq 0.05$) on the moisture, ash, fiber content, crude lipids, crude protein and total carbohydrate content of both treated and untreated *T. fuscatus*. The ash contents of *T. fuscatus* treated with NaCl and *C. aurantifolia* and the untreated *T. fuscatus* were the same and this agrees with results of Adeleke and Odedeji (2010). The high ash content (≥ 0.5 %) of *T. fuscatus* is an indication of good mineral content (Adeleke and Odedeji, 2010). The range of values obtained for the proximate compositions of *T. fuscatus* treated with NaCl and *C. aurantifolia* in the study corroborated the previous work of Adebayo-tayo and Ogunjobi (2008) who reported between 41.39 and 60.04% crude protein; 0.37 and 0.39% crude fibre, 3.4 and 10.5% ash and 20.2 and 22.5% moisture contents in *Tympanotonus* sp.

## Conclusion

These findings have shown that *T. fuscatus* is a source of good nutrients, but harboured pathogenic bacteria of public interest and treatment of *T. fuscatus* with 10% NaCl and *C. aurantifolia* either singly or in combination for 10 min holding time greatly reduced the microbial burden without significantly affecting the nutritional composition of the treated *T. fuscatus.*

## Conflict of Interest

Authors have declared that there is no conflict of interest reported in this study.

## References

Adebayo-Tayo, BC, Onilude AA, Ogunjobi AA & Adejoye DO 2006. Bacteriological and proximate analysis of periwinkles from two different creeks in Nigeria. *World Appl. Sci. J.*, 1(2): 87 - 91.

Adebayo-Tayo BC & Ogunjobi AA 2008. Comparative effects of oven drying and sun drying on the microbiological, proximate nutrient and mineral composition of *Tympanotonous* Spp. (Periwinkle) and *Crassostrea* spp. (Oyster). *J. Envtal. Agric. and Food Chem.*, 7(4): 2856 - 2862.

Adeleke RO & Odedeji JO 2010. Functional properties of wheat and sweet potato flour blends. *Pakistan Journal of Nutrition*, 9: 535 – 538.

Akinjogunla OJ, Inyang CU & Akinjogunla VF 2011. Bacterial species associated with anatomical parts of fresh and smoked Bonga fish (*Ethmalosa fimbriata*): Prevalence of cephalosporins. *Res. J. Microbi.*, 6(1): 87- 97.

Anbalagan M, Ganesh-Prabu P, Krishnaveni RE & Manivannan S 2014. Effect of sodium chloride (NaCl) on the bacterial load in chicken, mutton and beef meat samples in relation to meat spoilage. *Int. J. Res. in Zool.*, 4(1): 1 – 5.

AOAC 2005. *Official Method of Analysis* (18th ed). Association of Official Analytical Chemist International, Maryland, USA, 876p

Bob-Manuel FG 2012. A preliminary study on the population estimation of the periwinkles *Tympanotonus fuscatus* (Linnaeus, 1758) *and Pachymelania aurita* (Muller) at the Rumuolumeni mangrove swamp creek, Niger Delta, Nigeria. *Agric. and Biol. J. North Amer.*, 3(6): 265 - 270.

Cheng HY, Ye RC & Chou CC 2003. Increased acid tolerance of *Escherichia coli* O157:H7 by acid adaptation time and conditions of acid challenge. *Food Research International*, 36: 49 - 56.

Davidson PM & Taylor TM 2007. Chapter 33, Chemical Preservatives and Natural Antimicrobial Compounds. In: Doyle, M. P. and Larry R. Beuchat (eds.), *Food Microbiology - Fundamentals and Frontiers*, 3rd ed. ASM Press, Washington D.C. pp. 713 – 745.

Dorit RL, Walker WF & Barnes RD 1991. *Zoology*. Saunders College Publishing. Philadephia, p. 1008.

Ekanem EO & Adegoke GO 1995. Bacteriological study of West African clam (*Egeria radiata Lamarch*) and their overlying waters. *Food Microbiology*, 12: 381 - 385.

Ekanem EO & Otti BN 1997. Total plate count and coliform levels in Nigerian periwinkles from fresh and brackish water. *Food Control*, 8: 87 - 89.

Food & Nutrition Board 2007. Seafood Choices: Balancing Benefits and Risks. Food and Nutrition Board, Institute of Medicine, National Academies Press, Washington, DC.

Holt, JG, Krieg NR, Sneath PHA, Stately JT & Williams ST 1994. *St. Bergey's Manual of Determinative Bacteriology* (9th Edition). Baltimore, Williams and Wilkins, 787p.

Jatau SU, Emere MC, Addulsalam MS, Umar Y & Etangetuk NA 2014. Evaluation of bacterioplankton species in Calabar coastal water and Ship Ballat water. *Int. J. Fisheries and Aquatic Stud.*, 2(2): 09 - 14.

Man CMD 2007. Technological functions of salt in food products. In: Kilcast, D and Angus, F (eds): *Reducing Salt In Foods*. CRC Press LLc, Boca Raton Fl, pp. 157 - 173.

Mata L, Vives M & Vicente G 1994. Extinction of *Vibrio cholerae* in acidic substrata: contaminated fish marinated with lime juice (ceviche). *Revista de Biologia Tropical*, 42: 479 - 485.

Odu NN, Obafemi A & Njoku HO 2010. Comparative assessment of bacteriological quality and proximate composition of laboratory shucked and traditionally shucked tropical periwinkle (*Tympanotonus fuscatus*), department of microbiology, University of Port-Harcourt, Rivers - State, Nigeria. *Scientia Africana*, 9(1): 140 - 149.

Ogungbenle HN 7 Omowole BM 2012. Chemical, functional and amino acid composition of periwinkle (*Tympanotonus fuscatus Var. Radula*) meat. *Int. J. Pharmac. Sci. Rev. and Res.*, 13(2): 128 - 132.

Oranusi LS, Effiong ED & Duru NU 2018. Comparative study of microbial, proximate and heavy metal compositions of some gastropods, bivalve and crustacean seafood. *Afr. J. Clin. and Experi. Microbio.*, 19(4): 291 – 302.

Orjimelukwe PC, Ekong KS & Akachukwu D 2017. Effect of different processing methods on the nutrient composition and sensory properties of *Ethmalosa fimbriata. Amer. J. Agric. Sci.*, 4(5): 107 – 113.

Onyeagba RA & Isu NR 2006. *General Microbiology*, 2nd (ed) Crystal Publishers, Okigwe Nigeria, 600p.

Oyenekan JA 2010. The ecology of the Genus *Parchymelania* in Lagos Lagoon. *Archive of Hydrobiology*, 86: 515 - 522.

Pessu PO, Agoda S, Benson OB & Adeniran TR 2014. Effect of different processing methods on the meat of *Tympanotonus fuscatus*. *Scientia Africana*, 13(2): 47-52.

Ricke SC 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry science*, 82: 632 - 639.

Rodrigues A, Sandstorm A, Ca T, Steinsland H, Jensen H & Aaby P 2000. Protection from cholera by adding lime juice to food - results from community and laboratory studies in Guinea-Bissau, West Africa. *Trop. Med. and Int. Health*, 5: 418 - 422.

Skrivanova E, Molatova M, Houf K & Marounek M 2011. Inhibitory effect of organic acids on Acrobacters in culture and their use for control of *Acrobacter butzleri* on chicken skin. *Int. J. Microbio.*, 144: 367 - 371.

Soyiri IN, Agbogh HK & Dongdem YT 2008. A pilot microbial assessment of beef sold in the Ashaiman Market, a suburb of Accra Ghana. *J. Appl. Microbio*., 8: 72 – 74.

Wafaa MKB, Walaa AH & Amani FA 2011. Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. *Journal of American*, 7(9): 663 – 668.