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LOGARITHMIC AND PERCENTAGE REDUCTIONS OF BACTERIAL ISOLATES IN *GALATEA PARADOXA* TREATED WITH *CITRUS AURANTIFOLIA* AND NACL: PUBLIC HEALTH IMPLICATION AND NUTRITIONAL ASSESSMENT

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ABSTRACT
Shell fishes constitute a vital source of food for humans due to its high nutritional values. Bacteriological and nutritional assessments of *Galatea paradoxa* treated with *Citrus aurantifolia* and NaCl were determined using bacteriological and analytical protocols. The results revealed a reduction from 4.845 to 2.301 Log CFU/g in Total Heterotrophic Bacterial Counts (THBC) in *G. paradoxa* treated with 10% NaCl for 5 mins. The *G. paradoxa* treated with 7.5 % NaCl for 5 min had a reduction in Total Coliform Counts (TCC) ranging from 3.903 to 2.398 Log CFU/g, while Total Faecal Coliform Counts (TFC) in *G. paradoxa* treated with 5 % and 10 % for 10 min reduced by 99.99 %. There was 53.46% THBC reduction in *G. paradoxa* treated with 10% *C. aurantifolia* for 5 min; THBC in *G. paradoxa* treated with 10% *C. aurantifolia* for 10 min reduced by 79.36 %; THBC in *G. paradoxa* treated with 10 % equal concentrations of NaCl and *C. aurantifolia* decreased by 99.99 % within 10 min, while TCC in *G. paradoxa* treated with 7.5 % equal concentrations of NaCl and *C. aurantifolia* within 10 min of exposure had 99.99 % decrease. The predominant survived bacterial genera in treated samples were *Bacillus, Vibrio* and *Micrococcus*. There was insignificant difference ($p \geq 0.05$) between the nutritional compositions of treated and untreated samples. This study showed that *G. paradoxa* could be treated with *C. aurantifolia* and NaCl so as to avert possible foodborne diseases associated with consumption of this aquatic food.

Keywords: Bacteriological, *Citrus aurantifolia*, NaCl, *Galatea paradoxa,* Nutritional.

INTRODUCTION

An increase in population worldwide has resulted in a substantial increase in consumption of aquatic food such as *Galatea paradoxa* (Samya and Mohammed, 2006). *Galatea paradoxa* (Born 1778) are freshwater clams, bivalve and filter feeding mollusc that belong to order 'Veneroidea'; superfamily 'Tellinoidea' and family 'Donacidae' (Moses, 1990). These aquatic animals, without vertebral column, have two hinged calcareous shells that aid its protection and are endemic in West African countries such as Ghana, Nigeria and Cameroun (Etim and Brey, 1994; Villalobs and Elguezabel, 2001). The high nutritional values of shellfishes have triggered its increased consumption (Ekanem and Adegoke, 1995; Simopoulos, 2003). *Galatea paradoxa* constitute a vital source of food for humans due to its high protein content, low cholesterol content, significant amounts of omega-3-fatty acids (Ekpenyong *et al*., 2013), vitamins, iron, potassium, zinc, copper, manganese and selenium (Davies and Jamabo, 2016).

The *G. paradoxa* are a suitable bio-indicator of environmental pollution (Chiesa *et al*., 2018; Okon *et al*., 2020). They can accumulate human pathogenic organisms from sewage contaminated waters and also accumulate toxins in its soft tissues through feeding on toxic phytoplankton (Gram *et al*., 2002). The ingestion of contaminated soft tissues of *G. paradoxa* by humans may result in food-borne related diseases (Hathal *et al*., 2005). The soft tissue of *G. paradoxa* is consumed after frying, smoking, roasting, steaming or cooking (King, 2000; Villalobs and Elguezabel, 2001) and *G. paradoxa* also serves as a means of livelihood some dwellers in parts of Southern Nigeria.

Sodium chloride (NaCl) is one of the most extensively used food additives as a preservative, enhancing the flavour and enzymatic activities responsible for organoleptic parameters (Cheng *et al*., 2003; Silva *et al*., 2003), and improving water adsorption which aids in inhibiting growth of spoilage and pathogenic organisms (Lawrence *et al*., 2003; Man, 2007). The reduction of water activity due to addition of salt and presence of ions exerts osmotic pressure effects on the microorganisms, thus, increase the shelf life of the processed food (Anbalagan *et al*., 2014). The inadequacy of NaCl as a sole preservative in ready-to-eat and other food products has necessitated its combination with other preservation processes such as addition of chemicals, drying and osmotic dehydration.

The Food Safety and Inspection Service of the United States Department of Agriculture has approved the use of lime (*Citrus aurantifolia*) juice as a natural antimicrobial agent having been recognized as safe for its application in food (Skrivanova *et al*., 2011). The juice of *C. aurantifolia* has been reported to reduce the growth of some bacteria in family Enterobacteriaceae and its antibacterial activities has attributed to its low pH that can penetrate the bacterial membranes (Davidson and Taylor, 2007; Bradley *et al*., 2011). However, there is a need to evaluate natural occurring organic compound that can be applied as post-harvest treatment to *G. paradoxa*. Consequently, this study determined the effect of *citrus aurantifolia* and NaCl singly and in combination on bacterial loads and nutritional quality of *G. paradoxa.*

MATERIALS AND METHODS

Collection of Samples

Freshly harvested *G. paradoxa* were obtained from two major markets (Itam and Akpan Andem markets) in Uyo using sterile wide-mouth plastic containers and were transported to the Microbiology Laboratory, University of Uyo. The *G. paradoxa* was identified and confirmed by a Fish Taxonomist in the Department of Fisheries and Aquaculture, University of Uyo. *Galatea paradoxa* were extensively washed with sterile distilled water and rinsed with normal saline to remove all extraneous materials before shucking. The edible part was aseptically removed as described by APHA (1998) and was transferred into sterile containers for bacteriological and nutritional analyses.

Fig 1: *G. paradoxa*

Bacteriological Analysis of Samples

Ten (10) grams of each fleshy blended parts of *G. paradoxa* was aseptically suspended into 90 ml sterile distilled water, vigorously shaken to dislodge adhered bacteria and ten-fold serial dilutions were made to obtain dilutions 10^{-1} to 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 Agar (EMB) and the plates were aerobically incubated at 37°C for 24 hr. 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 NA plates and aerobically incubated at 37°C for 24 hr. The pure cultures of isolates were streaked onto NA 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 *C. aurantifolia* and NaCl on the Bacterial Loads of *G. paradoxa*

The *C. aurantifolia* juice was extracted using the method of Ndelekwute and Enyenihi (2017). Fleshy part of *G. paradoxa* 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, 10g of each fleshy part was blended, separately suspended into 90 ml sterile distilled water, vigorously shaken to dislodge adhered bacteria and dilutions were made to obtain 10^{-1} and 10^{-3}. One (1) ml of the aliquot was pour-plated in triplicate onto each plate of NA, MCA, EMB and incubated aerobically at 37 °C for 24 hr. The same procedure was carried out for combination of NaCl and *C. aurantifolia* at ratio of 1:1 (vol/vol). The same procedures were carried on control (*G. paradoxa* untreated with NaCl and *C. aurantifolia*). After incubation, the bacterial counts were recorded and mean, standard deviations were appropriately calculated.

Proximate Analysis of *G. paradoxa* treated with NaCl and *Citrus aurantifolia*

The moisture, lipid and ash contents of fleshy part of *G. paradoxa* samples were carried out using the methods of AOAC (2005). The fibre and protein contents were obtained by Kjeldahl's procedure and subsequently converted to crude protein by multiplying the values obtained with a protein conversion factor of 6.25. The energy content was calculated as follows: Energy Kcal 100g = (crude lipid x 8) + (crude protein x 2) + (CHO x 4), where CHO was carbohydrate contents of *G. paradoxa*. All determinations were done in triplicates and values obtained were expressed as mean ± standard deviation.

Statistical Analysis

The Statistical Package for Social Sciences (IBM SPSS Version 22.0) was used for data analysis. The significant difference ($p \leq 0.05$) between the nutritional compositions of *G. paradoxa* treated with NaCl / *Citrus aurantifolia* and the untreated *G. paradoxa* were determined using one-way Analysis of Variance (ANOVA).

RESULTS

The logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with NaCl are presented in Table 1. The results revealed a THBC reduction in *G. paradoxa* treated with 10% NaCl for 5 mins from 4.845 to 2.301 Log CFU/g, while THBC in *G. paradoxa* samples treated with 10% NaCl for 5 mins decreased from 4.845 to 1.146 Log CFU/g. The *G. paradoxa* treated with 7.5 % NaCl for 5 min had a TCC reduction ranging from 3.903 to 2.398 Log CFU/g; *G. paradoxa* treated with 7.5 % at 10 min had a reduction in TCC (3.903 to 1.041 Log CFU/g), while TFC in *G. paradoxa* treated with 5 % and 10 % for 10 min reduced by 99.99 % (Table 1)

The logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with *C. aurantifolia* are presented in Table 2. The

results showed 53.46% THBC reduction in *G. paradoxa* treated with 10% *C. aurantifolia* for 5 min, while THBC in *G. paradoxa* treated with 10% *C. aurantifolia* for 10 min reduced by 79.36 %. *G. paradoxa* treated with 7.5 % *C. aurantifolia* for both 5 min and 10 min did not have TCC, respectively; while *G. paradoxa* treated with 7.5 % *C. aurantifolia* for 5 min had no TFC (Table 2).

The logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with combination of NaCl and *C. aurantifolia* are presented in Table 3. The THBC in *G. paradoxa* sample treated with equal concentrations (10 %) of NaCl and *C. aurantifolia* decreased by 99.99 % within 10 min of exposure, while TCC and TFC in *G. paradoxa* treated with equal concentrations (vol / vol) of 7.5 % (NaCl and *C. aurantifolia*) within 10 min of exposure had 99.99 % decrease (Table 3).

The trends of occurrence of bacterial isolates from *G. paradoxa* treated with NaCl and *C. aurantifolia* singly and in combination are shown in Table 4. The results revealed that *B. subtilis, S. aureus, M. varians, E. coli, K. pneumoniae, V. cholerae* and *P. aeruginosa* survived in *G. paradoxa* treated with 5 % of NaCl or *C. aurantifolia* within 5 min of exposure. Although, *B. subtilis* were predominant in all the treated samples, but combination of 10% equal volume (vol / vol) of *C. aurantifolia* and NaCl killed the bacterial isolate.

The comparative analyses of proximate compositions of *G. paradoxa* treated with NaCl and *C. aurantifolia* singly and in combination are presented in Table 5. The highest moisture content of 67 ± 1 % was obtained from sample C_d (control), followed by sample C_b (*G. paradoxa* treated with 10 % *C. aurantifolia*) with 62 ± 1 %, while the lowest moisture content of 50 ± 2 % was obtained from sample C_c (*G. paradoxa* treated with equal volume of 10 % NaCl and 10 % *C. aurantifolia*). The ash content was highest in sample C_d with 3.950 ± 0.01% and lowest in sample C_c with 3.75 ± 0.03%. Samples C_c and C_a had the lowest fibre (0.023 ± 0.02 %) and protein (32.10 ± 0.25 %) content respectively, while the highest fibre (0.045 ± 0.01 %) and protein (33.229 ± 0.10 %) content was obtained in sample C_d. The crude lipid was highest in sample C_a with 1.360 ± 0.02 % and was lowest in sample C_c (1.290 ± 0.001%). The total carbohydrate was highest in sample C_a (62.700 ± 0.11%) and was lowest in sample C_c (58.606 ± 0.20 %). There was no statistically significant difference ($p > 0.05$) between the nutritional compositions of *G. paradoxa* treated with NaCl / *Citrus aurantifolia* and the untreated *G. paradoxa* (Table 5).

Table 1. Logarithmic and Percentage Reductions of Bacterial Loads in *Galatea paradoxa* Treated with NaCl

Exposure Time (mins)	Microbial Group	Concentration (%)	Plate Counts (CFU/g)	Log (CFU/g)	% Reduction	Log reduction
5	THBC	0	$7.0 \pm 0.4 \times 10^4$	4.845	NA	NA
		2.5	$6.4 \pm 0.7 \times 10^4$	4.806	0.80	0.04
		5.0	$4.5 \pm 0.1 \times 10^3$	3.653	24.60	1.19
		7.5	$3.0 \pm 0.7 \times 10^2$	2.447	48.88	2.37
		10	$2.0 \pm 0.3 \times 10^2$	2.301	52.51	2.54
	TCC	0	$8.0 \pm 0.1 \times 10^3$	3.903	NA	NA
		2.5	$3.9 \pm 0.3 \times 10^3$	3.591	7.994	0.31
		5.0	$5.6 \pm 0.8 \times 10^2$	2.748	29.59	1.16
		7.5	$2.5 \pm 0.5 \times 10^2$	2.398	38.66	1.51
		10	NG	NA	≥ 99.99	3.90
	TFC	0	$3.0 \pm 0.7 \times 10^2$	2.477	NA	NA
		2.5	$2.1 \pm 0.2 \times 10^2$	2.322	6.258	0.16
		5.0	$1.5 \pm 0.4 \times 10^2$	1.176	52.52	1.30
		7.5	NG	NA	≥ 99.99	2.48
		10	NG	NA	≥ 99.99	2.48
10	THBC	0	$7.0 \pm 0.4 \times 10^4$	4.845	NA	NA
		2.5	$5.2 \pm 0.4 \times 10^4$	4.716	2.663	0.13
		5.0	$3.9 \pm 0.3 \times 10^2$	3.591	25.88	1.25
		7.5	$2.1 \pm 0.2 \times 10^2$	2.322	52.07	2.52
		10	$1.4 \pm 0.1 \times 10^1$	1.146	76.35	3.70
	TCC	0	$8.0 \pm 0.1 \times 10^3$	3.903	NA	NA
		2.5	$4.5 \pm 0.1 \times 10^2$	2.653	32.03	1.25
		5.0	$2.1 \pm 0.2 \times 10^2$	2.322	40.50	1.58
		7.5	$1.1 \pm 0.0 \times 10^1$	1.041	73.33	2.86
		10	NG	NA	≥ 99.99	3.90
	TFC	0	$3.0 \pm 0.7 \times 10^2$	2.477	NA	NA
		2.5	$1.0 \pm 0.0 \times 10^2$	1.000	59.63	1.48
		5.0	NG	NA	≥ 99.99	2.30
		7.5	NG	NA	≥ 99.99	2.30
		10	NG	NA	≥ 99.99	2.30

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

Table 2 Logarithmic and Percentage Reductions of Bacterial Loads in *Galatea paradoxa* Treated with *Citrus aurantifolia*

Exposure Time (mins)	Microbial Group	Concentration (%)	Plate Counts (CFU/g)	Log (CFU/g)	% Reduction	Log reduction
5	THBC	0	$7.0 \pm 0.4 \times 10^4$	4.845	NA	NA
		2.5	$4.5 \pm 0.1 \times 10^4$	4.653	3.96	0.04
		5.0	$3.5 \pm 0.8 \times 10^3$	3.544	26.85	1.30
		7.5	$2.5 \pm 0.3 \times 10^2$	2.398	50.51	2.45
		10	$1.8 \pm 0.7 \times 10^2$	2.255	53.46	2.59
	TCC	0	$8.0 \pm 0.1 \times 10^3$	3.903	NA	NA
		2.5	$3.3 \pm 0.2 \times 10^3$	3.591	9.839	0.38
		5.0	$2.8 \pm 0.4 \times 10^2$	2.748	37.30	1.46
		7.5	$1.2 \pm 0.7 \times 10^1$	1.079	72.35	2.82
		10	NG	NA	≥ 99.99	3.90
	TFC	0	$3.0 \pm 0.7 \times 10^2$	2.477	NA	NA
		2.5	$1.4 \pm 0.3 \times 10^2$	2.146	13.36	0.16
		5.0	$1.0 \pm 0.0 \times 10^1$	1.000	59.63	1.30
		7.5	NG	NA	≥ 99.99	2.48
		10	NG	NA	≥ 99.99	2.48
10	THBC	0	$7.0 \pm 0.4 \times 10^4$	4.845	NA	NA
		2.5	$2.0 \pm 0.4 \times 10^3$	3.301	31.87	1.54
		5.0	$2.4 \pm 0.2 \times 10^2$	2.380	50.88	2.47
		7.5	$2.0 \pm 0.4 \times 10^1$	1.301	73.15	3.54
		10	$1.0 \pm 0.0 \times 10^1$	1.000	79.36	3.85
	TCC	0	$8.0 \pm 0.1 \times 10^3$	3.903	NA	NA
		2.5	$2.8 \pm 0.4 \times 10^2$	2.447	37.30	1.46
		5.0	$1.0 \pm 0.0 \times 10^1$	1.000	74.38	2.90
		7.5	NG	NA	≥ 99.99	3.90
		10	NG	NA	≥ 99.99	3.90
	TFC	0	$3.0 \pm 0.7 \times 10^2$	2.477	NA	NA
		2.5	NG	NA	≥ 99.99	2.48
		5.0	NG	NA	≥ 99.99	2.48
		7.5	NG	NA	≥ 99.99	2.48
		10	NG	NA	≥ 99.99	2.48

Keys: 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 3: Logarithmic and Percentage Reductions of Bacterial Isolates in *Galatea paradoxa* Treated with NaCl and *Citrus aurantifolia*

Exposure Time (mins)	Microbial Group	Concentration (%)	Plate Counts (CFU/g)	Log (CFU/g)	% Reduction	Log reduction
5	THBC	0	$7.0 \pm 0.3 \times 10^4$	4.845	NA	NA
		2.5	$3.9 \pm 0.1 \times 10^4$	4.591	5.243	0.25
		5.0	$2.1 \pm 0.1 \times 10^3$	3.322	31.43	1.52
		7.5	$1.1 \pm 0.7 \times 10^2$	2.041	57.87	2.80
		10	$1.0 \pm 0.3 \times 10^2$	2.000	58.72	2.85
	TCC	0	$8.0 \pm 0.4 \times 10^3$	3.903	NA	NA
		2.5	$4.5 \pm 0.2 \times 10^2$	2.653	32.03	1.25
		5.0	$1.0 \pm 0.3 \times 10^2$	2.000	48.76	1.90
		7.5	NG	NA	≥ 99.99	3.90
		10	NG	NA	≥ 99.99	3.90
	TFC	0	$3.0 \pm 0.7 \times 10^2$	2.477	NA	NA
		2.5	NG	NA	≥ 99.99	2.48
		5.0	NG	NA	≥ 99.99	2.48
		7.5	NG	NA	≥ 99.99	2.48
		10	NG	NA	≥ 99.99	2.48
10	THBC	0	$7.0 \pm 0.3 \times 10^4$	4.845	NA	NA
		2.5	$1.8 \pm 0.4 \times 10^3$	3.255	32.62	1.59
		5.0	$1.6 \pm 0.6 \times 10^2$	2.204	54.51	2.64
		7.5	$1.0 \pm 0.3 \times 10^2$	2.000	58.72	2.85
		10	NG	NA	≥ 99.99	4.85
	TCC	0	$8.0 \pm 0.4 \times 10^3$	3.903	NA	NA
		2.5	NG	NA	≥ 99.99	3.90
		5.0	NG	NA	≥ 99.99	3.90
		7.5	NG	NA	≥ 99.99	3.90
		10	NG	NA	≥ 99.99	3.90
	TFC	0	$3.0 \pm 0.7 \times 10^2$	2.477	NA	NA
		2.5	NG	NA	≥ 99.99	2.48
		5.0	NG	NA	≥ 99.99	2.48
		7.5	NG	NA	≥ 99.99	2.48
		10	NG	NA	≥ 99.99	2.48

Keys: THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Coliform Counts; NG: No Growth; NA: Not Applicable; CFU: Colony Forming Unit; Log: Logarithmic.

Table 4: Occurrence of Bacterial Isolates in *G. paradoxa* treated with NaCl and *C. aurantifolia*

Bacterial Isolates	UTD	A (5 mins)		A (10 mins)		B (5 mins)		B (10 mins)		A + B (5 mins)		A + B (10 mins)		Total
	0%	5%	10%	5%	10%	5%	10%	5%	10%	5%	10%	5%	10%	No
B. subtilis	+	+	+	+	+	+	+	+	+	+	+	-	-	11
S. aureus	+	+	-	+	-	+	-	+	-	+	-	-	-	6
M. varians	+	+	+	+	-	+	-	+	-	+	-	-	-	7
S. enterica	+	-	-	-	-	+	-	+	-	-	-	-	-	3
S. pyogenes	+	-	-	-	-	+	-	-	-	-	-	-	-	2
E. coli	+	+	-	+	-	+	-	+	-	+	-	-	-	6
K. pneumoniae	+	+	-	-	-	+	-	+	-	-	-	-	-	4
E. aerogenes	+	-	-	-	-	-	-	-	-	-	-	-	-	1
V. cholerae	+	+	-	+	-	+	-	+	-	+	-	+	-	7
E. faecium	+	-	-	-	-	+	+	-	-	-	-	-	-	3
P. aeruginosa	+	+	-	+	-	+	-	+	-	-	-	-	-	5
Total	11	7	2	6	1	10	2	8	1	5	1	1	0	55

Keys: UTD: Untreated; A: NaCl; B: *Citrus aurantifolia*; A + B: Sodium Chloride + *Citrus aurantifolia*; +: Present; -: Absent.

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Table 5: Proximate Compositions of *G. paradoxa* treated with NaCl and *Citrus aurantifolia*

Sample	Treatment	Percentage / mm ± S.D						
		Moisture Content	Ash Content	Fiber Content	Crude Lipid	Crude Protein	Total CHO	Calorie Value (kcal)
C_a	NaCl	61±2	3.800±0.01	0.040±0.01	1.360±0.002	32.100±0.25	62.700±0.11	391.468±0.001
C_b	*C. aurantifolia*	62±1	3.800±0.02	0.041±0.01	1.322±0.000	32.222±0.22	62.615±0.15	391.228±0.002
C_c	NaCl + *C. aurantifolia*	50±2	3.750±0.03	0.023±0.02	1.290±0.001	32.631±0.15	58.606±0.20	376.558±0.000
C_d	Control	67±1	3.950±0.01	0.045±0.01	1.320±0.002	33.229±0.10	61.951±0.30	392.600±0.004

Each value represents the means of triplicate and standard deviation. CHO: Carbohydrate, ANOVA ($p > 0.05$)

DISCUSSION

Even though shellfishes, *G. paradoxa,* are substantially nutritious and have become an increasingly significant source of inexpensive proteins and other nutrients essential for maintenance of healthy body of a large section of world population, nevertheless, shellfishes harbour some pathogenic microorganisms attributable to poor hygienic conditions of the water bodies from where they are obtained (Adebayo-tayo *et al*., 2006; Oranusi *et al*. 2018).

In our study, a high THBC, TCC and FCC were obtained from *G. paradoxa* and these high bacterial loads substantiated the results of Ekanem and Adegoke (1995) and Oranusi *et al.* (2018) who discretely observed unacceptable bacterial loads in shell fishes. The high bacterial loads from *G. paradoxa* not treated with NaCl and *C. aurantifolia* in our study conform with the findings of Antai (1998) and Tonbarapagha *et al*. (2018) who obtained high microbial loads in shell fishes, but these findings contradicted the report of Udoh *et al*. (2017) who reported low bacterial loads from *G. paradoxa* in Cross River, Nigeria. Our findings showed that bacterial loads from *G. paradoxa* in Uyo, Akwa Ibom State, exceeded the acceptable limits for shellfishes as specified by FDA (1991) and ICMSF (2005). The high bacterial loads in the shell fishes, *G. paradoxa,* could be attributed to poor handling and processing in the markets (Odu *et al*., 2010; Akinjogunla *et al.,* 2011).

In this study, logarithmic and percentage reductions in bacterial loads (THBC, TCC and TFC) in *G. paradoxa* treated with different concentrations of NaCl and *C. aurantifolia* singly or in combination for ≥ 10 mins were obtained. The decrease in logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with different concentrations (5 to 10%) of NaCl agrees with Soyiri *et al.* (2008) and Anbalagan *et al.* (2014) who reported that NaCl concentrations between 7.5 and 10 % eliminated all pathogenic bacteria from shell fishes. A medium containing 10 % NaCl has been reported as unfavourable medium for proliferation of pathogenic micro-organisms (Onyeagba and Isu, 2006; Anbalagan *et al*. 2014). Similarly, studies have showed that NaCl removed water from food products by osmosis and as NaCl content in food increased its water content also decreased, thus, leading to plasmolysis of cell walls of pathogenic micro-organisms (Anbalagan *et al.* 2014; Orjimelukwe *et al.* 2017). This study revealed 79.36 % THBC reduction in *G. paradoxa* treated with 10% *C. aurantifolia* for 10 min. The reduction in bacterial loads to an acceptable level for human consumption in our study corroborated the findings of Rodrigues *et al*. (2000) and similarly agrees with the results of Mata *et al*. (1994) who reported extinction of some bacterial isolates in acidic medium containing lime (*C. aurantifolia*) juice.

The eleven bacterial genera obtained from *G paradoxa* not treated with NaCl and *C. aurantifolia* were *Staphylococcus*, *Micrococcus, Salmonella*, *Streptococcus*, *Escherichia, Klebsiella*, *Bacillus, Enterobacter, Vibrio, Enterococcus* and *Pseudomonas*. The isolation of *S. pyogenes*, *V. cholerae*, *E. coli, S. enterica* and *S. aureus* in our study substantiated the reports of Udoh *et al.* (2017) who obtained these bacterial isolates from *G paradoxa* in Cross River, Nigeria.

Our findings revealed 33.229 % protein content in untreated *G. paradoxa* (control) and this value was lower than 47.0 % obtained by Ehigiator and Akise (2016) in Delta State, but our findings agrees with Ivon and Eyo (2018) who reported 32.10 % protein content in *G. parad*oxa from Calabar River, Nigeria. The moisture content in *G. paradoxa* was high in this study and this was in conformity with Zhu and Bai (2007). The high moisture content in shellfish has been attributed to the quantity of water absorbed into their cells from the external environment (Davies and Jamabo, 2016). A high ash content was discretely obtained from the untreated and *G. paradoxa* treated with NaCl and *C. aurantifolia*, and this concurs with results of Adeleke and Odedeji (2010). The ash content greater than 0.5 % has been reported as an indication of good mineral content in food (Adeleke and Odedeji, 2010).

CONCLUSION

This study showed that *G. paradoxa,* harboured some pathogenic bacteria of public interest and its treatment with equal concentration (vol/vol) of 10 % *C. aurantifolia* and NaCl singly or in combination for 10 min holding time will avert possible foodborne diseases associated with consumption of this aquatic food.

REFERENCES

Adebayo-Tayo, B.C., Onilude, A. A., Ogunjobi, A. A. and Adeoye, D. O. (2006). Bacteriological and proximate analysis of periwinkles from two different creeks in Nigeria. *World Applied Science Journal*, 1(2): 87 - 91.

Adeleke, R. O. and Odedeji, J. O. (2010). Functional properties of wheat and sweet potato flour blends. *Pakistan Journal of Nutrition*, 9: 535 – 538.

Akinjogunla, O. J., Inyang, C. U. and Akinjogunla, V. F. (2011). Bacterial species associated with anatomical parts of fresh and smoked Bonga fish (*Ethmalosa fimbriata*): Prevalence of cephalosporins. *Research Journal of Microbiology*, 6(1):87- 97.

Anbalagan, M., Ganesh Prabu, P., Krishnaveni, R. E. and Manivannan, S. (2014). Effect of sodium chloride (NaCl) on the bacterial load in chicken, mutton and beef meat samples in relation to meat spoilage. *International Journal of Research in Zoology*, 4 (1): 1 – 5.

Antai, S. P. (1998). Study of the microbial flora of Nigeria mussel species. *International Journal of Food Microbiology*, 6: 259 - 261.

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

APHA (American Public Health Association). (1998). Standard methods for the examination of water and wastewater. 16th Edition, Washington D.C.

Bradley, E. M., Williams, J. B., Schilling, M. W., Coggin, P. C and Crist, C. (2011). Effects of sodium lactate and acetic acid preservatives on the quality and sensory characteristics of hot-

boned pork sausage patties. *Meat Science,* 88: 145 - 150.

Chiesa, L. M., Nobile, M., Malandra, R., Pessina, D and Panseri, S. (2018). Food safety traits of mussels and clams: distribution of PCBs, PBDEs, OCPs, PAHs and PFASs in sample from different areas using HRMS-Orbitrap and modified QuEChERS extraction followed by GC-MS. *Food Additives and Contaminants,* 35: 5.

Cheng, H. Y., Ye, R. C. and Chou, C. C. (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, P. M. and T. M. Taylor. (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.

Davies, I. C. and Jamabo, N. A. (2016). Determination of mineral contents of edible parts of shellfishes from Okpoka creeks in Rivers State, Nigeria. *International Journal of Fisheries and Aquaculture Research*, 2 (2): 10 - 18.

Ehigiator, F. A. R. and Akise, O. G. (2016). Proximate, amino acid and mineral composition of wild and cultured fresh water clam (*Egeria radiate*). *Nigerian Journal of Agriculture Food and Environment*, 12 (2): 103 - 108.

Ekanem, E. O. and Adegoke, G. O. (1995). Bacteriological study of West African clam (*Egeriaradiata Lamarch*) and their overlying waters. *Food Microbiology*, 12: 381 - 385.

Ekpenyong E, Williams I.O, Osakpa U. U. (2013). Variation in the proximate, energy and mineral compositions of different body parts of *Macrobrachium macrobranchion* (Prawn). *Journal of Food Research*, 2 (2): 150 – 156.

Etim, L and Brey, T. (1994). Growth, productivity and significance for fishery of the bivalve *Egariaradiata* (*Donacidae)* of live bivalve molluscs. pp. 1 - 19.

FDA, (1991). Sanitation of shellfish. growing areas considering and seafood safety (F.E. Ahmed). National Academic Press. Washington DC. *Food and Environment*, 12 (2): 103 - 108.

Gram, L., Ravn, L. and Rasch, M. 2002. Food spoilage interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 78: 79-97.

Hatha, A. A. M., Christi, K.C., Singh, R. and Kumar, S. (2005). Bacteriology of the fresh water bivalve clam *Batissa violacea* (Kai) sold in the Suva Market. *The South Pacific Journal of National Science*, 23: 48 – 50.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stately, J. T. and Williams, S. T. (1994). *St. Bergey's Manual of Determinative Bacteriology* (9th Edition). Baltimore, Williams and Wilkins. 787p.

ICMSF *International commission on microbiological specificat ions for foods* (2005). *Microorganisms in Foods*. 2nd ed, Kluwer Academic/ Plenum Pub. New York. pp. 84 – 86.

Ivon, E. A. and Eyo, V. O. (2018). Proximate composition and mineral contents of edible part of four species of shellfishes from the Calabar River, Nigeria. *Annual Research and Review in Biology*, 26 (1): 1 – 10.

King, R. P. (2000). Population structure, growth performance and mortality rates of the freshwater clam *G. paradoxa* Born 1778, in Nun River, Nigeria. *Archive of Fisheries and Marine Research,* 48(1) :21 - 30.

Lawrence, T. E., Dikeman, M. E., Stephens, J. W., Obuz, E. and Davis, J. R. (2003). In Situ Investigation of the calcium-induced proteolytic and salting-in mechanisms causing tenderization in calcium-enhanced muscle. *Meat Science*, 66: 69 - 75.

Man, C. M. D. (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. and Vicente, G. (1994). Extinction of *V. cholerae* in acidic substrata: contaminated fish marinated with lime juice (ceviche). *Revista de Biologia Tropical*, 42: 479 - 485.

Moses, S. B. (1990). Growth, biomass, mortality, production and potential yield of the West African clam, *Egeria radiata* (Lamarck) (Lamellibranchia, Donacidae) in the Cross-River State, Nigeria. *Hydrobiologia*, 196: 1–15.

Ndelekwute, E. K. and Enyehini, G. E. (2017). Lime juice as a source of organic acids for growth and apparent nutrient digestibility of broiler chickens. *Journal of Veterinary Medicine and Surgery*, 1: 1 – 5.

Odu, N. N., Obafemi, A. and Njoku, H. O. (2010): Comparative assessment of bacteriological quality and proximate composition of laboratory shucked and traditionally shucked tropical periwinkle (*Tympanotonus fuscatus*). *Scientia Africana*, 9 (1): 140 - 149.

Okon, M. U., Inyang, C. U. and Akinjogunla, O. J. (2020). Bacterial isolates from bivalve clams (*G paradoxa*, Born 1778): Occurence, multi-drug resistance, location of antibiotic resistance marker and plasmid profiles. *South Asian Journal of Research in Microbiology* ,7(3): 35 – 46.

Orjimelukwe, P. C., Ekong, K. S. and Akachukwu, D (2017). Effect of different processing methods on the nutrient composition and sensory properties of *E. fimbriata. American Journal of Agricultural Science*, 4(5): 107 – 113.

Onyeagba, R. A. and Isu, N. R. (2006). *General Microbiology*, 2nd (ed) Crystal Publishers, Okigwe Nigeria, 600p.

Oranusi 1, S., Effiong, E. D. and Duru, N. U. (2018). Comparative study of microbial, proximate and heavy metal compositions of some gastropods, bivalve and crustacean seafood. *African Journal of Clinical and Experimental*

Microbiology, 19 (4): 291 – 302.

Rodrigues, A., Sandstorm, A., Ca, T., Steinsland, H., Jensen, H. and Aaby. P. (2000). Protection from Cholera by adding lime juice to food - results from community and laboratory studies in Guinea-Bissau, West Africa. *Tropical Medicine and International Health*, 5: 418 - 422.

Samya, H. M. and Mohamed, S. Y. (2006). Proximate evaluation of some economical seafood as a human diet and as an alternative prospective valuable of fish meal. *Journal of Fisheries and Aquatic Science.* 11 (1): 12 - 27.

Silva, J. G., Morais, H. A. and Silvestre, M. P. C. (2003). Comparative study of the functional properties of bovine globin isolates and sodium caseinate. *Food Research International,* 36: 73 – 80.

Simopoulos, A. P. (2003). Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. *World Review of Nutrition and Dietetics*, 92: 1 - 22.

Skrivanova, E., Molatova, M., Houf, K and Marounek, M. (2011). Inhibitory effect of organic acids on Acrobacters in culture and their use for control of *Arcobacter butzleri* on chicken skin.*International Journal Microbiology*, 144: 367 - 371.

Soyiri, I. N., Agbogh, H. K and Dongdem, Y. T. (2008). A pilot microbial assessment of beef sold in the Ashaiman Market, a suburb of Accra Ghana. *Journal of Applied Microbiology*. 8: 72 – 74.

Tonbarapagha, K., Douye, V. Z. and Deborah, A. (2018). "Assessing the hygienic status of processed fresh water clam (*Galatea paradoxa*) in Yenagoa Metropolis, Bayelsa State, Niger Delta, Nigeria." *American Journal of Food Science and Technology*, 6 (5): 219 - 222.

Udoh, D. I., Udo, I. U. and Udoh, E. I. (2017). Microbiological analysis of the freshwater clam from Cross River, Nigeria. *Nigeria Journal of Agriculture, Food and Environment*, 13 (3): 59 – 64.

Villalobos, L. B. and Elguezabal, L. A. (2001). Microbiological quality of the bivalves *Pinctadaimbricate* commercialized in Cumana, Venezuela. *Food Technology. Acta Cientifica Venezolona*, 52: 55 – 61.

Zhu, Q. and Bai, R. (2007). Comparison of biological characteristics between cultured and wild crab (*Eriocheir sinensis*) *Jiangsu Journal of Agricultural Science*, 23: 218 - 223.

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