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Microbiological Changes in Freshwater Prawn (*Macrobrachium vollenhovenii*, Herklots 1857) Stored in Ice

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ABSTRACT

This study aimed to isolate and characterize spoilage causing organisms of *Macrobrachium vollenhovenii* as well as determine the shelf-life under ice storage. Fresh prawns and those exposed to two ice treatments: Direct Contact with Ice (DCI) and Without Contact with Ice (WCI) were evaluated for changes in a 10 day period. Aerobic plate and coliform counts range of 6-7 and 5-7 log₁₀ cfu g⁻¹ were observed for DCI, 6-7 and 3-6 log₁₀ cfu g⁻¹ were recorded for WCI and 3-5 and 4-6 log₁₀ cfu g⁻¹ for the fresh prawn. Both ice treatments significantly (p<0.05) curtailed the presence of *Proteus mirabilis*, *Escherichia coli*, *Enterobacter aerogenes* and *Salmonella* sp. after 6 days of storage. Ice treatments were unable to control the psychrophilic microbes *Staphylococcus* sp., *Bacillus cereus* and *Pseudomonas aeruginosa*. The population of these organisms particularly *Pseudomonas aeruginosa* increased significantly (p<0.05) with storage days. The shelf-life of freshwater prawn as determined by microbiological data is 7 days of ice storage in this study. *Pseudomonas aeruginosa* promotes spoilage of freshwater prawn in the absence of H₂S and indole producing bacteria *Proteus mirabilis* and *Escherichia coli*.

Key words: *Macrobrachium vollenhovenii*, shelf-life, ice, *Pseudomonas aeruginosa*, indole

INTRODUCTION

Macrobrachium vollenhovenii continues to receive tremendous attentions as it supports very important local fisheries in Nigeria and other regions of Africa (Jayachandran, 2001; Akintola *et al.*, 2009b). Also, the safety and quality of the prawn at different biomes had been evaluated for microbes and reported to be suitable for consumption (Akintola *et al.*, 2009a).

Ice against traditional smoking for fish products is becoming more popular. However, irregular supply of electricity in the country hampers the use of ice as a means of preserving fish products in spite of the high profile of ice globally as the cheapest and commonest method for prawn preservation (Kirschnik *et al.*, 2006).

Prawns offer quality protein, low saturated fat, may contain Omega-3 (Ω-3) fatty acids and contribute to cardiovascular stability of adults as well as children's growth and development (FDA, 2007). Rapid spoilage due to higher water content and other issues mentioned in Abu-Bakar *et al.* (2008) are important concern and affect shelf-life of the prawn.

The shelf-life depends on the numbers and types of microorganisms, mainly bacteria, initially present and their subsequent growth as well as natural sources (Abu-Bakar *et al.*, 2008). Shelf-life

reported for whole *M. rosenbergii* stored on ice, included 3 days (Nip and Moy, 1988), 8 days (Lindner *et al.*, 1988), 7-10 days (Kirschnik *et al.*, 2006) 14 days (Abu-Bakar *et al.*, 2008).

Lalitha and Surendran (2006) reported reduction in bacterial load after 5 days due to the inability of some of the bacterial species to survive and/or grow at low temperatures. Studies on the impact of ice on the qualities and safety of other freshwater prawn of genus *Macrobrachium* are rare in spite of their widely acknowledged status as species of ecological and economic importance to many fisheries.

The objective of this study was to evaluate the impact of ice in controlling the population of microbes in *Macrobrachium vollehovenii* and to determine the shelf-life. In this light microbial composition of freshly collected *M. vollehovenii* were investigated against the prawns exposed to two ice treatments.

MATERIALS AND METHODS

This study was carried out within the period of 12th March to 12th June, 2010 in the Laboratories of the Fisheries and Microbiology Departments of the Lagos State University, Ojo.

Collection of sample: Live *Macrobrachium vollehovenii* with mean weight of 30±25 g were purchased from fishers from Badagry Creek at the Topo landing station in hours of 7.00-8.00 a.m. Samples were packed into a sterile Thermocool® boxes containing chlorinated water (5 ppm) and transported within 1 h to the laboratory in the Department of Fisheries, Lagos State University. The whole prawns were then washed in deionized water and after which the water in the Thermocool® box was drained. The samples were confirmed killed after 20-25 min of no contact with water.

Samples were aseptically distributed randomly into two groups of prawn stored with Direct Contact with Ice (DCI) in ratio of 1:1 (w/w) in Thermocool® box labelled A while the samples without direct contact with ice were placed 0.006 mm thick polythene bags, 150 g prawn/bag following Kirschnik *et al.* (2006) and thereafter placed in the Thermocool® box labelled B.

Melted ices in each box were replaced after 12 h and drained regularly through the tap on the Thermocool® box. Five prawns were withdrawn for microbial analyses the first day and thereafter every two days of storage (day 0, 2, 4, 6, 8, 10) from the two experimental set up under room temperature of 27-30°C while the fresh prawns served as control.

Microbiological analysis: Twenty five gramme of the prawn muscle tissues were aseptically taken and crushed in a mortar with a pestle. Prawn homogenates were prepared by adding 9 mL of sterile distilled water to 1 g of the crushed samples. Thereafter, 0.1 mL each of the homogenized solutions was inoculated using spread plate method into nutrient and MaConkey agar and incubated at 25°C for 24 to 36 h. The total viable count was determined on the nutrient agar and coliform on the MaConkey agar. The dilution out was 10⁻⁴ using pour method. After incubation the colonies were counted using colony forming unit and cfu g⁻¹ was calculated.

Characterizations and identification of isolate: In order to identify each isolate, pure cultures were examined for cultural and morphological characteristics based on their colour, shape and pigmentation, by Gram staining. The bacterial isolates were identified using Bergey's Manual of Systematic Bacteriology (Ludwig and Klenk, 2001).

Statistical analysis: Analysis of variance was performed using statistical Package for Social Scientist (SPSS, 2006) version 15. One way ANOVA was conducted to test differences in the mean values between fresh prawns and each of the treatments in five replications. $p < 0.05$ was considered statistically significant.

RESULTS

Flora on fresh prawn: The total aerobic bacterial counts on freshly caught wild whole freshwater *Macrobrachium vollehovenii* ranged from 3-5 \log_{10} cfu g^{-1} . A total of seven bacterial species were isolated from the fresh prawn (Table 1). The Isolates were mainly Gram-negative bacteria with *Escherichia coli* being the most dominant (61%). Indole and H_2S producing bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* and *Proteus mirabilis* and *Salmonella* sp. collectively constituted 29% of the isolates. Only two Gram-positive rod and cocci, *Bacillus cereus* and *Staphylococcus* sp. were isolated

Bacteria flora on prawn stored in ice: The impact of the two icing treatments on the African river prawn were alike significantly ($p < 0.05$) in terms of ability to cause the absence of bacteria which were present in the freshly caught prawn (Table 1 and Fig. 1). *Proteus* sp. and *Enterobacter aerogenes* were not detected in the two ice treatments by day 6 whereas, both treatments inhibit the presence of *Salmonella* sp. at 8 days storage, respectively.

The population of *Pseudomonas aeruginosa* increased significantly ($p < 0.05$) after 6 days with population counts of 10^6 - 10^7 cfu g^{-1} while the H_2S and the other indole producing bacteria, *Escherichia coli* were not detected. This suggested the dominant role of *Pseudomonas aeruginosa* in promoting spoilage of freshwater prawn. Although, in this study *Staphylococcus* sp. showed psychrophillic attributes and icing treatments were not able to reduce the population of the organism. Population of Gram-positive rod and coccus *Bacillus cereus* and *Staphylococcus* sp. increased with storage days in ice.

Table 1: Microflora in fresh *Macrobrachium vollehovenii* and succession during ice storage

Microorganism	Treatments	Storage (days) (% occurrence)					
		0	2	4	6	8	10
<i>Staphylococcus</i> sp.	DCI	20	17	15	14	14	14
	WCI	22	10	15	16	15	13
<i>Proteus</i> sp.	DCI	7	3	3	0	0	0
	WCI	5	3	3	0	0	0
<i>Bacillus</i> sp.	DCI	15	18	16	16	16	15
	WCI	20	17	17	17	15	15
<i>Escherichia coli</i>	DCI	15	7	5	3	0	0
	WCI	17	10	8	0	0	0
<i>Pseudomonas aeruginosa</i>	DCI	26	35	42	57	68	71
	WCI	24	33	47	60	70	70
<i>Enterobacter aerogenes</i>	DCI	5	5	3	0	0	0
	WCI	8	4	5	0	0	0
<i>Salmonella</i> sp.	DCI	10	8	6	10	0	0
	WCI	8	10	5	7	0	0

Values are not significantly different between treatments, $p > 0.05$

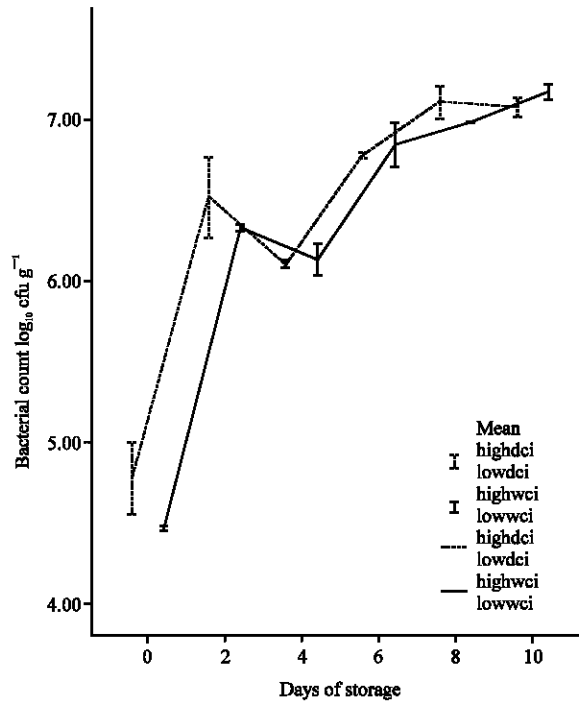


Fig. 1: Changes in bacterial counts (\pm SD) on *Macrobrachium vollehovenii* during 10 days storage in two ice treatments

DISCUSSION

The total aerobic bacterial counts on fresh *Macrobrachium vollehovenii* were within values (about $5 \log_{10} \text{ cfu g}^{-1}$) previously reported for farmed *Macrobrachium rosenbergii* (Leitao and Rios, 2000) but below $7.00 \log_{10} \text{ cfu g}^{-1}$ specified by the International Commission on Microbiological Specification for Foods (ICMSF, 1986). The total bacteria counts were not significantly different ($p > 0.05$) between treatments.

The percentage composition of indole and H_2S producing bacteria were considerable lower in the fresh prawn nevertheless, they have been implicated as agents of spoilage in food (Leitao and Rios, 2000). The isolation of two Gram-positive rod and cocci, is similar to findings of Lalitha and Surendran (2006) with lower population of Gram-positive bacteria in farmed fresh *Macrobrachium rosenbergii*.

The presence of *Escherichia coli* in the fresh prawn and not reported for farmed *M. rosenbergii* in Brazil (Leitao and Rios, 2000) shows the influence of prevailing environment on the bacteria composition in muscle of freshwater prawn as averred by Gomez-Gil *et al.* (1998). Bello-Olusoji *et al.* (2008) reported the presence of *Salmonella* sp. and *Escherichia coli* in *M. vollehovenii* and stated that they are habitat selective. *Salmonella* sp. and *Escherichia coli* are indicator microorganisms of fecal pollution which are not indigenous to the aquatic environments (Papadopoulou *et al.*, 2007). *Staphylococcus* sp. is reported to be part of the normal human and animal microflora and may be found in aquatic systems polluted by sewage (Papadopoulou *et al.*, 2007). Incidence of human activities particularly the slaughtering and processing of pork in the banks of Badagry Creek was observed by Akintola *et al.* (2009a).

The impact of the two icing treatments on the African river prawn were alike significantly ($p < 0.05$) in terms of ability to cause the absence of bacteria which were present in the fresh prawn.

Proteus sp. and *Enterobacter aerogenes* were not detected in the two ice treatments by day 6. Whereas, both treatments inhibit the presence of *Salmonella* sp. at 8 days storage, respectively indicating differential abilities of bacteria to withstand icing conditions as reported by Miyamoto-Shinogara *et al.* (2000). *Proteus* sp., *Enterobacter* sp. and *Salmonella* sp. are reported to not being able to tolerate cold storage (Nimrat *et al.*, 2008).

Nimrat *et al.* (2005) note that *Staphylococcus* sp., *Bacillus cereus* and *Pseudomonas aeruginosa* are present in the spermatophores of fresh black tiger shrimp (Nimrat *et al.*, 2008) showed strong resistant to ice storage conditions in this study. The significant increase ($p < 0.05$) in the population of these group after 6 days while the H_2S and indole producing *Escherichia coli* were not detected suggested dominant role of *Pseudomonas aeruginosa* in promoting spoilage of freshwater prawn. The most prolific organisms during the ice storage of fish were found in significant numbers at the end of storage (Kirschnik *et al.*, 2006). Psychrophilic *Pseudomonas* sp. is a primary cause of spoilage irrespective of initial microflora on prawns stated Abu-Bakar *et al.* (2008).

According to Papadopoulou *et al.* (2007), *Staphylococcus* sp. are not able to propagate in competition with fishes natural microflora. Population of Gram-positive rod and coccus *Bacillus cereus* and *Staphylococcus* sp increased with storage days in icing in view of their psychrophilic nature (Lalitha and Surendran, 2006).

Ice treatments as a means of ensuring food safety and quality of prawns needs further investigation since the population of *Staphylococcus* sp, *Bacillus cereus* and *Pseudomonas aeruginosa* were not controlled by this means. These organisms have been implicated as promoting food borne diseases (Le Loir *et al.*, 2003; Archer, 2004) and are of health concerns as regards food safety globally. Both *Staphylococcus* sp. and *Bacillus cereus* are diarrhoeal and emetic toxins producers that cause serious illness and fatalities in human consequent to consuming food contaminated with the toxins.

Further study need to be explored as regards the status of *Staphylococcus* sp. in relations to the type of strains particularly, Staphylococcal Enterotoxins (SE) producing types in the freshwater prawn. Le Loir *et al.* (2003) stated the need for better understanding of the interactions between *S. aureus* and the food matrix and of the mechanisms of SE production in foodstuffs. *Bacillus cereus* is a common pathogen in foods. However, the cfu numbers may not be sufficient to draw conclusion about the status of the safety and quality of the food substance. Attention should rather be placed to the toxicity and the level of cereulide in the food.

A wide range of pathogens isolated in this study are related to human and animal interface particularly *Staphylococcus* sp., *Salmonella* sp. and *Escherichia coli*. This indicated the importance of defining this interface as a critical control point in the management of food safety in prawn from the wild. Also, it implies the need to define and enforce acceptable limits of interaction between human and aquatic environments. The practice of using ice as a means of achieving safe and quality prawn is adequate when spatial and temporal distance is short between source of capture and consumption. The population of the psychrophilic *Pseudomonas* sp. festered with increasing days of storage, the prawns are however safe when not consumed beyond the 7 days of storage with ice.

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