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BACTERIOLOGICAL QUALITY AND MICROBIAL HEALTH RISKS ASSOCIATED WITH FRESH-FROZEN AND DRIED-PROCESSED READY-TO-SELL PRAWNS (*MACROBRACHIUM ROSENBERGII*)

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ABSTRACT

This study set out to determine the bacteriological quality and the microbial health risks associated with ready-to-sell prawns. Fresh-frozen prawns were obtained from supermarkets and dried-processed prawns were obtained from local markets in Akure, Nigeria (n = 120). Levels of *E. coli*, faecal coliforms, *Shigella*, *Salmonella* and *Staphylococcus aureus* in the samples were determined using standard microbiological methods. Probabilities of infection (P_i) associated with exposure to *Salmonella* and *Shigella* were determined using beta-Poisson model and for *S. aureus* exponential model was adopted. Mean level of *Salmonella* was 0.9 $\log_{10}$ CFU 100 g^{-1} in fresh-frozen prawns whereas mean levels of *Shigella* in fresh-frozen and dried-processed prawns were 4.4 and 4.5 $\log_{10}$ CFU 100 g^{-1} respectively. Probability of infection with exposure to a single CFU of *Salmonella*, *Shigella* and *S. aureus* was predicted at 1.6×10^{-7}, 1.9×10^{-4} and 7.6×10^{-8} respectively. This study demonstrates that the levels of bacterial contamination of the prawns and the risks per serving per year were unacceptable.

Keywords: Food safety, human health, foodborne pathogens, *Macrobrachium rosenbergii,* dose-response models

INTRODUCTION

Seafood can form a relevant contributor of a healthy human diet and one of the most common seafood is freshwater prawn (*Macrobrachium rosenbergii*). Prawns are rich in protein, vitamins, omega-3 fatty acids and some essential nutrients required in human diet **(Kakara *et al*., 2016)**. The presence of bacterial pathogens in prawns suggest contamination and potential health risk to consumers **(Rosmini *et al*., 2004)**. This is because most seafood bioaccumulate spoilage organisms (that may be responsible for rapid deterioration of seafood quality) and pathogens (that may be responsible for outbreak of infectious diseases) from their aquatic environment **(Polo *et al*., 2014; Olalemi *et al*., 2021)**. Prawns are classified as perishable food and they require quick storage since rapid microbial spoilage may occur because of the inherent free amino acids and other soluble non-nitrogenous substances in prawns that can serve as digestible nutrients for microbial growth **(Zeng *et al*., 2005; Gokoglu and Yerlikaya, 2008)**. Generally, freshwater prawns deteriorate due to poor hygienic conditions, improper handling, inappropriate processing, preservation and storage condition.

In Nigeria, fresh-frozen or dried-processed ready-to-sell prawns are available in major supermarkets and local markets. These prawns may be contaminated with pathogens that may cause foodborne illness. Contamination of seafood is largely a reflection of the microbial quality of their growing waters before harvesting and handling or storage after harvesting **(Campos and Lees, 2014; Olalemi *et al*., 2016)**. Globally, *Salmonella*, *Shigella* and *Staphylococcus aureus* are among the most significant causes of outbreaks of foodborne illness and *E. coli* is widely used as an indicator of faecal contamination in seafoods and their growing waters and they also show close relationship with health hazards associated with gastrointestinal symptoms **(Olalemi *et al*., 2021)**. The probability of experiencing illness as a result of consuming contaminated food may be described as the number of people that manifest symptoms of an infection within a group of people exposed to pathogens in the food. Hazard identification, dose-response models, exposure assessment and risk characterization are useful for understanding the risks posed by pathogens in food **(Lee *et al.*, 2015)**.

This study was aimed at determining the bacteriological quality of fresh-frozen and dried-processed ready-to-sell prawns in Akure. The objectives of the study were to investigate the levels of bacterial contamination that may be unacceptable in prawns with potential human health hazard; determine the probability of infection that may occur as a result of consuming the prawns; and suggest essential control measures that may be required in order to reduce associated risks.

MATERIAL AND METHODS

Collection and preparation of prawn samples

Fresh-frozen prawns were purchased from Ceci supermarket and Shoprite shopping mall while dried-processed prawns were purchased from three different local markets, namely Oja Oba, Isikan and NEPA markets in Akure, Nigeria. A total of eight (8) batches containing three (3) samples from each of the five (5) outlets were obtained over a period of three months (February, March and April, 2019) (n = 120). On each sampling occasion, prawns were placed in sterile polyethylene bags and cool box and transferred to the laboratory for analysis within one hour. The prawns were washed and macerated using mortar and pestle and about 25 g was added into 75 ml of sterile nutrient broth to make a stock. The stock sample was diluted serially up to fourth dilution.

Enumeration of bacteria in the prawn samples

The concentrations of *E. coli*, faecal coliforms, *Shigella*, *Salmonella* and *Staphylococcus aureus* in the fresh-frozen and dried-processed prawns were determined using standard microbiological method. About 1 ml aliquot from the second and third dilutions from the stock were aseptically pour-plated on the freshly prepared selective media: Membrane Lauryl Sulphate agar (MLSA), Eosin Methylene Blue (EMB) agar, Membrane Faecal Coliform (*m*-FC) agar and Mannitol Salt agar (MSA). Also, about 1 ml aliquot from the stock were aseptically pour-plated on the freshly prepared *Salmonella*-*Shigella* agar (SSA). Agar plates were incubated at 37°C for 24 hours (MLSA, EMB, SSA, MSA) and 44°C for 24 hours (*m*-FC). Colonies were counted, calculated and expressed as colony-forming units (CFU) 100 g^{-1} of prawn samples.

Probability of foodborne illness from human exposure to the prawn samples

Dose-response models were used to evaluate the probability of infection as a result of consuming prawns contaminated with *Salmonella*, *Shigella* or *S. aureus*. The beta-Poisson model was utilized to determine the probability of infection associated with exposure to *Salmonella* and *Shigella* in the prawns.

$$Pi = 1 - \left(1 + \frac{N}{\beta}\right)^{-\alpha} \qquad (1)$$

where:

P_i = Probability of infection;
α, β = Parameters defining the dose-response curve; and
N = exposure (colony forming unit)

There are little information on dose-response models for *S. aureus* in food. To this end, we applied exponential model described by Lee *et al.* (2015) to evaluate the probability of infection as a result of consuming prawns contaminated with *S. aureus*. We also estimated the probable level of enterotoxin produced by *S. aureus* in the prawns assuming that enterotoxin dose of ≤ 1.0 µg (1000 ng) may be reached when the concentration of *S. aureus* exceed 10^5 cfu/g in food (FDA, 2012).

$$Pi = 1 - \exp\,(-rN) \qquad (2)$$

where:

P_i = Probability of infection;
r = Parameters defining the dose-response curve; and
N = exposure (colony forming unit).

Furthermore, we evaluated the annual probability of infection as a result of consuming fresh-frozen and dried-processed prawns contaminated with *Shigella*, *Salmonella* and *S. aureus*.

$$P_A = 1 - (1 - Pi)^{365} \quad (3)$$

where:

P_A = Annual probability of infection; and
P_i = Probability of infection

A single serving of food containing microbial contaminants may result into illness when consumed. Human health risks due to exposure to pathogens in food is proportional to the level and virulence of the pathogen as well as the volume of the contaminated food consumed. According to US Department of Agriculture, one can consume more than 200 g of cooked seafood in a week. In Nigeria, prawns ranging from 57 g to 146 g averaging around 100 g are cooked with varieties of soups (such as spinach, broccoli, lady finger, melon), meals (such as rice, spaghetti, salad, pasta, spicy meat skewer 'suya', beans cake 'akara', steamed bean pudding 'moi moi', yam porridge and plantain porridge) that may be consumed in a single meal. In some cases, prawns are ground into powder and are used as condiments in preparation of food. Although, cooking may lead to inactivation of the pathogens in food, but regrowth may occur if foods are left for certain period of time before consumption. In this study, 100 g will be assumed as the goal for monitoring the probability of infection that may occur as a result of consuming the prawns.

Statistical analysis

Data were transformed to $\log_{10}$, then examined using general descriptive statistics. Further analyses were undertaken using one way analysis of variance (ANOVA) and means were separated by Duncan's New Multiple Range test using SPSS version 22, at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Detection of bacteria in the prawn samples

The concentrations of *E. coli*, faecal coliforms, *Shigella*, *Salmonella* and *Staphylococcus aureus* in the fresh-frozen and dried-processed prawn samples varied (Figure 1). The mean concentration of *E. coli* that was 3.9 Log_{10} CFU 100 g^{-1} in fresh-frozen prawns and 5.4 Log_{10} CFU 100 g^{-1} in dried-processed prawns were lower compared to those observed by **Disegha and Onuegbu (2018)** where the authors reported high concentration of *E. coli* in fresh prawns collected from different local markets in Port Harcourt, Nigeria. This observation further supports the hypothesis that processing techniques such freezing and drying are important in minimizing microbial loads, enhancing the keeping quality and preservation of foods. *E. coli* is widely used as an indicator of faecal contamination in food and water and it exhibit close relationship with health hazards associated with diarrhoeal diseases **(Olalemi *et al*., 2021)**. It is important to note that the source of the faecal contamination may likely be from the prawn's growing waters, wash water or during handling and other processing stages. The presence of faecal coliforms in food or water is an indication of contamination with feces-related bacteria. Although, studies have demonstrated that faecal coliforms have less predictive value for enteric viruses or other pathogens **(Olalemi and Akinwumi, 2022)**, they are still very useful microbial tool for monitoring the sanitary quality of food and water and represent one of the classic bacterial indicators of the presence of faecal pollution in the environment **(Sinclair *et al*., 2012)**. The mean concentration of faecal coliforms that was 5.2 Log_{10} CFU 100 g^{-1} in fresh-frozen prawns and 6.1 Log_{10} CFU 100 g^{-1} in dried-processed prawns may likely be from the prawn's growing waters, wash water or during handling and other processing stages.

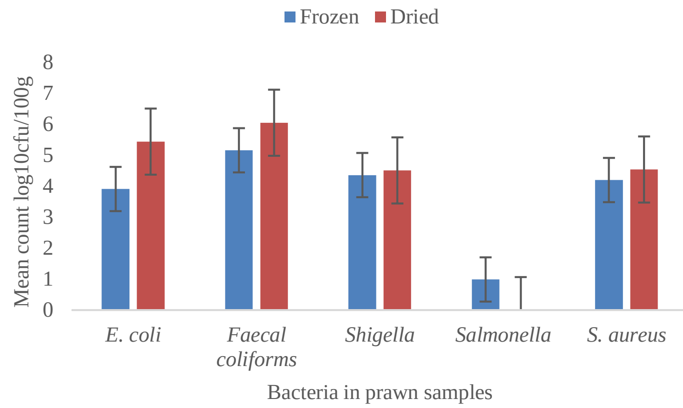

Figure 1 Mean count of bacteria in fresh-frozen and dried-processed prawns

Shigella is responsible for bacillary dysentery or shigellosis with symptoms ranging from mild to severe and acute diarrhoea. Some strains are capable of producing Shiga toxins and enterotoxins and humans are the primary reservoir of the pathogen. Its transmission occurs through direct or indirect contact with water contaminated with faeces of infected persons. *Shigella* infection is highly communicable, because ingestion of as few as 10 viable organisms may initiate an infection **(FDA, 2012)**. Outbreaks have been associated with person-to-person in crowded or unhygienic environments and with ingestion of contaminated food and water. Prawns may become contaminated during handling or preparation by an infected food handler **(Iwamoto *et al*., 2010)**. The level of *Salmonella* in the dried-processed ready-to-sell prawns were below detection limit. This observation may be not be unconnected with the intensive drying process of the prawns after harvesting. **Huss (1993)** suggested that seafoods may pose minimal health risks to the consumers when well-cooked except for cross contamination. The level of *Salmonella* in the fresh-frozen ready-to-sell prawns detected in three out of the eight batches may be as a result of cross contamination during the processing of the prawns prior or after freezing. The presence of *Salmonella* in food may result into non-typhoidal salmonellosis or typhoid fever depending on the type of species or strain responsible for the infection. Symptoms of non-typhoidal salmonellosis include nausea, diarrhoea, vomiting and fever, while symptoms of typhoid fever include diarrhoea, headache and high fever **(FDA, 2012)**.
The mean concentration of *Staphylococcus aureus* was 4.2 Log_{10} CFU 100 g^{-1} in fresh-frozen prawns and 4.5 Log_{10} CFU 100 g^{-1} in dried-processed prawns. The higher levels of *S. aureus* detected in the dried-processed prawns compared to those in the fresh-frozen prawns may likely be as a result of the ability of the bacteria to survive for long periods in dry state **(FDA, 2012)**. Furthermore, *S. aureus* are part of the normal flora of human skin, hair and nose and their presence in food is an indication of poor hygiene during food processing and handling and this may present certain health hazards because the organism has the potential of producing heat-stable enterotoxins that may cause food poisoning with symptoms such as abdominal pain, vomiting, nausea and diarrhoea when ingested by humans **(Lindqvist *et al*., 2002)**.

Probability of foodborne illness from human exposure to the prawn samples

The beta-Poisson model showed that the values of 'P_i' associated with exposure to *Salmonella* ranged from zero to 1.1×10^{-4} (levels of 1,202 CFU) in fresh-frozen prawns. Similarly, the values of 'P_i' associated with exposure to *Shigella* ranged from 9.4×10^{-2} (levels of 676 CFU) to 6.9×10^{-1} (levels of 316,227 CFU) in fresh-frozen prawns and 4.6×10^{-1} (levels of 19,952 CFU) to 5.9×10^{-1} (levels of 74,131 CFU) in dried-processed prawns. The exponential model showed that the values of 'P_i' associated with exposure to *S. aureus* ranged from 3.3×10^{-4} (levels of 4,365 CFU) to 2.8×10^{-3} (levels of 37,153 CFU) in fresh-frozen prawns and 1.5×10^{-3} (levels of 19,952 CFU) to 4.8×10^{-3} (levels of 63,095 CFU) in dried-processed prawns (Table 1). The estimated probable level of enterotoxin produced by *S. aureus* ranged from 0.4 to 3.7 ng in fresh-frozen prawns and 2.0 to 6.3 ng in dried-processed prawns (Table 2). The results from this study indicated that all the prawns obtained from local and supermarkets in Akure do not have high microbiological quality. This observation is in agreement with findings reported on ready-to-eat (RTE) frozen seafoods processed in Ijora-olopa **(Okonko *et al*., 2008)** and fresh prawns sold in local markets in Port Harcourt **(Disegha and Onuegbu, 2018)**.

Table 1 Probability of infection (P_i) associated with exposure to *Salmonella*, *Shigella* and *S. aureus* in prawns

Batch number	P_i associated with *Salmonella*		P_i associated with *Shigella*		P_i associated with *S. aureus*	
	Fresh-frozen prawns	Dried-processed prawns	Fresh-frozen prawns	Dried-processed prawns	Fresh-frozen prawns	Dried-processed prawns
1	ND	ND	9.4×10^{-2}	4.6×10^{-1}	8.2×10^{-4}	2.8×10^{-3}
2	1.6×10^{-5}	ND	1.9×10^{-1}	5.0×10^{-1}	3.3×10^{-4}	2.7×10^{-3}
3	1.9×10^{-4}	ND	4.5×10^{-1}	5.9×10^{-1}	2.8×10^{-3}	4.6×10^{-3}
4	1.1×10^{-4}	ND	4.7×10^{-1}	5.2×10^{-1}	1.6×10^{-3}	4.8×10^{-3}
5	ND	ND	4.7×10^{-1}	5.2×10^{-1}	1.6×10^{-3}	2.2×10^{-3}
6	ND	ND	5.9×10^{-1}	4.8×10^{-1}	1.6×10^{-3}	1.9×10^{-3}
7	ND	ND	6.8×10^{-1}	4.8×10^{-1}	1.6×10^{-3}	1.5×10^{-3}
8	ND	ND	6.9×10^{-1}	5.4×10^{-1}	1.2×10^{-3}	2.3×10^{-3}

Key: *Salmonella*; $\alpha = 0.175$; $\beta = 1110000$ **(Hornick *et al.*, 1966)**; *Shigella*; $\alpha = 0.2099$; $\beta = 1120$ **(Crockett *et al.*, 1996)**; *S. aureus*; $r = 7.64 \times 10^{-8}$ **(Lee *et al.*, 2015)**; ND = Non Detect

Table 2 Estimated enterotoxin (ng) produced by *S. aureus* in prawns

Batch number	Estimated enterotoxin (ng)	
	Fresh-frozen prawns	Dried-processed prawns
1	1.1	3.7
2	0.4	3.5
3	3.7	6.0
4	2.0	6.3
5	2.1	2.8
6	1.7	2.5
7	2.1	2.0
8	1.5	3.0

Key: ng = nanogram

The annual risk of *Salmonella* infection due to ingestion of contaminated fresh-frozen prawns ranged from zero to 0.07 whereas the annual risk of *Shigella* infection due to ingestion of contaminated fresh-frozen and dried-processed prawns was 1.00 (Figure 2). In addition, the annual risk of *S. aureus* infection due to ingestion of contaminated fresh-frozen and dried-processed prawns ranged from 0.11 to 0.64 and 0.42 to 0.83, respectively (Figure 3). The probability of infection with exposure to a single CFU of *Salmonella*, *Shigella* and *S. aureus* was predicted at 1.6×10^{-7}, 1.9×10^{-4} and 7.6×10^{-8} respectively.

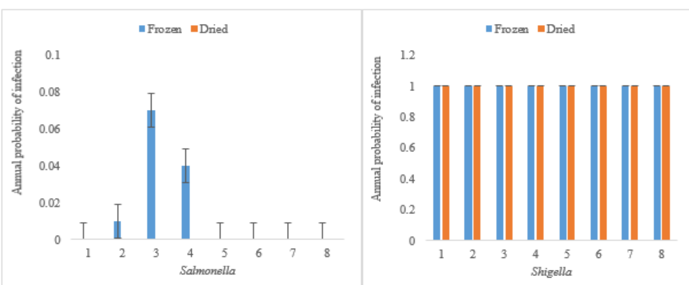

Figure 2 'P_A' associated with exposure to *Salmonella* (A) and *Shigella* (B) in fresh-frozen and dried-processed prawns

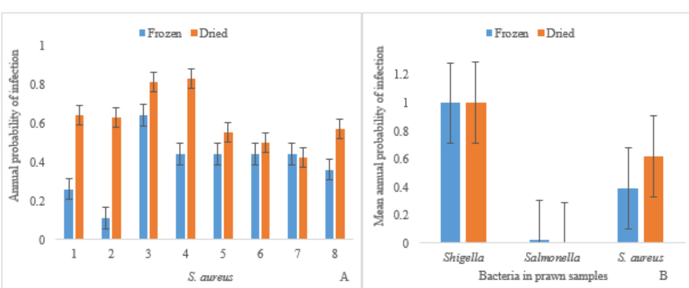

Figure 3 'P_A' associated with exposure to *S. aureus* (A) and mean 'P_A' associated with the bacteria (B) in fresh-frozen and dried-processed prawns

To compare the level of risk of infection associated with the bacteria, the mean annual probability of infection as a result of consuming fresh-frozen and dried-processed prawns contaminated with *Shigella*, *Salmonella* and *S. aureus* was determined. The mean annual probability of *Shigella* infection due to ingestion of the prawns was 1.0. The mean annual probability of *Salmonella* infection due to ingestion of fresh-frozen prawns was 0.02, while the mean annual probability of *S. aureus* infection due to ingestion of fresh-frozen prawns was 0.39 and dried-processed prawns was 0.62 (Figure 3).

Akure is the largest city and capital of Ondo State in Nigeria with a population of about 668,000 in the urban area. Assuming that there was uniform risk and if each person in the population was exposed to 1000 CFU of *Salmonella* or *Shigella* or *S. aureus* in the prawns once in a year, the annual cases of infection associated with exposure to *Salmonella* would be predicted to be 100 cases, those associated with exposure to *Shigella* would be predicted to be 125,000 cases and those associated with exposure to *S. aureus* would be predicted to be 50 cases. The probabilities of *S. aureus* infection due to ingestion of prawns determined in this study was higher than those observed by **Lee *et al.* (2015)** where the authors estimated the probabilities of *S. aureus*-related foodborne illness per person per day to be 7.84×10^{-10} and 2.24×10^{-9} for natural and processed cheese, respectively. In addition, enterotoxin dose of ≤ 1.0 µg (1000 ng) may be reached when the concentration of *S. aureus* exceed 10^5 cfu g^{-1} (5 log_{10} cfu g^{-1}) in food. At this level the food or food product may be injurious to human health when consumed and ingestion of 100 to 200 ng of enterotoxin may cause symptoms in sensitive or immunocompromised individuals **(FDA, 2012)**. In this study, the estimated probable levels of enterotoxin produced by *S. aureus* were not above 3.7 ng in fresh-frozen prawns and 6.3 ng in dried-processed prawns. This observation suggests that ingestion of the prawns may not cause symptoms but continuous consumption of the prawns may lead to accumulation of enterotoxins to levels sufficient enough to cause symptoms.

The occurrence of enteric organisms in the prawns suggests faecal pollution of the harvesting and/or overlaying waters. The sources of the faecal contamination of the harvesting and/or overlaying waters may likely originate from untreated or partially treated wastewater, faecal matter of animals or runoff from farmlands **(Kostyla *et al.*, 2015; Olalemi *et al.*, 2020)**. The detection of *S. aureus* in the prawns suggests poor sanitation and quality related factors at different stages of handling, transportation and processing by the seafood vendors, including temperature abuse especially through intermittent power supply and inadequate monitoring of food standards by government agencies. Essential control measures that may be required in order to eliminate or reduce associated risks of foodborne illness from human exposure to the prawns may include the protection and improvement of harvesting and/or overlaying waters from faecal contamination from point and non-point sources, maintenance of hygienic conditions during harvesting of the prawns, ensuring that wash waters are not faecally impacted, hand washing with soap under running water before, during and after prawn processing, prevention of cross contamination by maintenance of proper hygiene of utensils and board surfaces, refrigeration must be employed when storing prawns over long periods, application of heat during preparation of prawns to eliminate any form of pathogen and consumption of prawns immediately after preparation. In addition, a certificate of microbiological quality of prawns from producers and suppliers may be useful. These essential control measures may offer improved ways to protect human health.

CONCLUSION

The levels of bacterial contamination of the prawns were unacceptable and may pose significant health risks to consumers. Whilst the suggested essential control measures may be useful in human health protection, the exponential and beta-Poisson models were valuable tools for determining the probabilities of infection and health hazards associated with ingestion of the prawns. The findings of this study will help policy and decision makers including government agencies in the development of food standards and risk management of foodborne illnesses associated with fresh-frozen and dried-processed ready-to-sell prawns.

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