Full Length Research Paper

Comparative studies and microbial risk assessment of different Ready-to-Eat (RTE) frozen sea-foods processed in Ijora-olopa, Lagos State, Nigeria

Okonko, Iheanyi Omezuruike¹*, Ogunjobi, Adeniyi Adewale², Fajobi, Enobong Aloysius³, Onoja Bernard Anyebe¹, Babalola, Eunice Temilade¹, and Adedeji Adetola Olamide¹

¹Department of Virology, University of Ibadan College of Medicine, University college Hospital (UCH), Ibadan, Nigeria. WHO Regional Reference Polio Laboratory, WHO Collaborative Centre for Arbovirus Reference and Research, WHO National Reference Centre for Influenza.

²Enviromental Microbiology and Biotechnology Unit, Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria.

³Department of Basic Sciences, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria.

Accepted 30 June, 2008

This study reports the comparative studies and microbial risk assessment of different frozen seasfoods processed in Ijora-olopa, Lagos State, Nigeria. Different varieties of popularly consumed frozen sea-foods bought from different processing shops in Ijora-olopa, Lagos were microbiologically analyzed for the presence of microorganisms. Total plate counts, enterobacteriaceae counts and Salmonella-Shigella (SS) counts were enumerated using Plate Count Agar (PCA), Eosin Methylene Blue (EMB) Agar and Salmonella-Shigella Agar (SSA), respectively. The total counts for all the processed frozen seafood products ranged between 1.08×10^2 to 2.86×10^4 CFU/ml. These were generally high exceeding the limit of 1.0×10^2 CFU/ml. The coliform count ranged between 0.76×10^2 and 1.36×10^4 cells. The Salmonella-Shigella (SS) count ranged between 0.26×10^2 and 0.96×10^4 cells. Seventeen (17) isolates were characterized from the samples on PCA with percentage of occurrence of different microorganisms characterized as follows: *Bacillus cereus* (29.4%), *Enterobacter aerogenes* (29.4%), *Salmonella* sp. (17.6%), *Flavobacterium* sp. (11.8%), *Micrococcus* sp. (5.9%), and *Staphylococcus auerus* (5.9%). Consumption of some of this water and the seafood product processed with these water samples available in the market should be discouraged.

Key words: Food safety, HACCP, foodborne pathogens, microbiological quality, frozen seafood.

INTRODUCTION

Food security is a complex issue, which is influenced by a number of factors. Increasing national agricultural production alone cannot improve food security. The food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (Edema et al., 2005). In Nigeria, there is a large number of public frozen seafood processing services distributed along the country, where a considerable number of people buy their frozen seafood product daily. Serious consequences relating to national productivity and development can arise from lack of hygiene and sanitation in such outlets. There have been several reports on the health risks associated with the consumption of processed seafood, ranging from allergic reactions, stomach and intestinal cancerous growths, a general degeneration of peripheral cellular tissues, to gradual breakdown of the digestive and excretive systems in a statistically high per-

^{*}Corresponding author. E-mail: mac2finney@yahoo.com.

centage of people exained. Few of these reports however, have looked at the likely risks from a microbiological food safety point of view (Edema et al., 2005).

According to Higgins (2007), anyone who works in food safety sooner or later discovers that one of the most valuable tools for prevention is simply reading about and understanding how past outbreaks have occurred. Using major and frequently famous or at least newsworthy outbreaks, Phyllis (2007) illustrates how critical factors come together to produce tragic and largely preventable results. Modern microbes often team up with old practices, short sighted decisions, or current consumer trends to produce an outbreak. Recipes that do not include an adequate final cooking step have become increasing popular with consumers and can be a significant source of food-borne illness.

Biological contaminants such as bacteria, viruses, fungi, protozoa and helminthes constitute the major cause of food-borne diseases with varying degrees of severity, ranging from mild indisposition to chronic or lifethreatening illness, or both. In developing countries, such contaminants are responsible for food borne diseases such as cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellosis, shigellosis, typhoid fever, brucellosis, amoebiasis and poilomyletis (Edema et al., 2005).

Ready-to-eat (RTE) processed frozen seafood product consisting of peeled shrimps, headless shrimps, jumbo prawn, croaker filets, sole filets, fish steaks, calamari cleaned, red mullets, lobster and crab, fish fingers, seafood mix, and seafood skewers, packed in take-away packs and polythene bags and sold at frozen temperature are becoming popular in Nigeria markets. Mainly washing procedures, processing and storage temperature influences the shelf-life of these products.

The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication (Edema and Omemu, 2004). The Hazard Analysis Critical Control Point (HACCP) concept is used to identify microbiological vulnerable points in the food production process and processing, to determine the most appropriate methods of control to be applied, usually such methods as improved handling techniques, monitoring of temperature and more intensive supervision (Edema and Omemu, 2004).

This investigation aimed to evaluate the incidence of biological pathogens particularly microbes in our processed frozen seafood products, with a view to providing potential approaches to improve their quality, consumer safety and sanitary standard for the processing plants. Therefore, this current study reports on the comparative studies and microbial risk assessment of different Readyto-Eat (RTE) frozen sea-foods processed in Ijora-olopa, Lagos State, Nigeria

MATERIALS AND METHODS

Processed frozen seafood products such as peeled shrimps, head-

less shrimps, jumbo prawn, croaker filets, sole filets, and calamari cleaned were purchased from various processing shops, located at different areas of ljora-olopa, Lagos. Duplicate samples of each of the processed frozen seafood products were used in this study. Each sample that was bought from the different shops was kept in different polythene bags and brought to the laboratory for analysis. The five different samples are jumbo prawn (A), peeled shrimps (B), croaker filets (C), sole filets (D), and head-on shrimps (E).

These processed frozen seafood products were rinsed thoroughly with sterile distilled water. The sterile distilled water washing of the different processed frozen seafood products were then inoculated separately on different Plate Count Agar (PCA), Eosin Methylene Blue (EMB) Agar and Salmonella-Shigella Agar (SSA) plates and the plates were incubated at 37°C for 24 - 48 h.

Alongside the microbial evaluation of the sterile distilled water washing, the washed processed frozen seafood products were also assessed for bacterial growth. This was done by mutilating and macerating the processed frozen seafood products into small pieces and a piece was introduced to the surface of the prepared Plate Count Agar (PCA), Eosin Methylene Blue (EMB) Agar and Salmonella-Shigella Agar (SSA) plates. Different Plate Count Agar (PCA), Eosin Methylene Blue (EMB) Agar and Salmonella-Shigella Agar (SSA) plates were used for different samples and the plates were incubated at 37°C for 24 - 48 h for evidence of growth. Pure isolates of resulting growth were identified using biochemical methods as described by Jolt et al. (1994).

RESULTS

The total counts, coliform count and Salmonella-Shigella count of different processed frozen seafood samples is shown in Table 1. The total counts for all the processed frozen seafood products were generally high exceeding the limit of 1.0×10^2 CFU/ml (Table 1). The counts ranged between 1.08×10^2 to 2.86×10^4 cells (Table 1). The total count indicates that Sample D has the highest count (2.86×10^4); followed by sample A (1.96×10^4) and B (1.27×10^4) while sample C and E had a low count of 2.04×10^2 and 1.08×10^2 respectively (Table 1).

The coliform count ranged between 0.76×10^2 and 1.36×10^4 cells, also exceeding the limit of zero CFU/ml (Table 1). This indicates that sample D also has the highest count (1.36×10^4), followed by Sample A (0.87×10^4) and B (0.56×10^4) while sample E had the lowest coliform count (0.76×10^2), followed by sample C (1.12×10^2) as shown in Table 1.

The Salmonella-Shigella (SS) count ranged between 0.26×10^2 and 0.96×10^4 cells, also exceeding the limit of 1.0×10^2 CFU/ml (Table 1). This also indicates that Sample D had the highest SS count (0.96×10^4), followed by sample A (2.24×10^2) and sample B (1.86×10^2) while sample C and E had a low SS count of 0.35×10^2 and 0.26×10^2 respectively (Table 1).

Table 2 shows the bacteria isolated and identified from the surface of the different processed frozen seafood products, *Bacillus cereus* [5 (29.4%)], *Enterobacter aerogenes* [5 (29.4%)] and *Salmonella* sp. [3 (17.6%)] were most frequently isolated being present in almost all the seafood products, followed by *Flavobacterium* sp. [2 (11.8%)] and this was only isolated from sample A and E. *Micrococcus* sp. [1 (5.9%)] was only isolated from sample

| Sample | Total count (CFU/mI) | Coliform count (CFU/ml) | Salmonella-Shigella count (CFU/ml) |
|--------|-------------------------|----------------------------|---------------------------------------|
| А | 1.96 x 10 ⁴ | 0.87 x 10 ⁴ | 2.24 x 10 ² |
| В | 1.27 x 10 ⁴ | 0.56 x 10 ⁴ | 1.86 x 10 ² |
| С | 2.04 x 10 ² | 1.12 x 10 ² | 0.35 x 10 ² |
| D | 2.86 x 10 ⁴ | 1.36 x 10 ⁴ | 0.96×10^4 |

 Table 1. Total counts, Salmonella-Shigella (SS) counts and most probable number (MPN) of coliform/100 ml of different processed frozen seafood samples.

Sample **A** is jumbo prawn, **B** is peeled shrimps, **C** is croaker filets, **D** is sole filets, and **E** is head-on shrimps.

 0.76×10^2

 1.08×10^2

Table 2. The different types of bacteria found on the surface of the different processed frozen seafood products.

| | Frequency | Frozen seafood samples | | | | |
|------------------------|------------|------------------------|---|---|---|---|
| Bacterial Isolates | No. (%) | Α | в | С | D | Е |
| Bacillus cereus. | 5 (29.4) | + | + | + | + | + |
| Enterobacter aerogenes | 5 (29.4) | + | + | + | + | + |
| Flavobacterium sp. | 2 (11.8) | + | - | - | - | + |
| Micrococcus sp. | 1 (5.9) | - | + | - | - | - |
| Salmonella sp | 3 (17.6) | + | + | - | + | - |
| Staphylococcus auerus | 1 (5.9) | - | - | - | - | + |
| Total | 17 (100.0) | 4 | 5 | 2 | 3 | 4 |

Sample A is jumbo prawn, B is peeled shrimps, C is croaker filets, D is sole filets, and E is head-on shrimps.

B and *Staphylococcus auerus* [1(5.9%)] only from sample E.

F

B. cereus and *E. aerogenes* were found in all seafood products, *Salmonella* sp. were also found in all seafood products except sample C and E while *S. aureus* was found only on sample E (croaker fillet). *Flavobacterium* sp. was found on sample A and E [prawn and shrimps], while *Micrococcus* sp. was found also in sample B. These results are shown in Table 2.

DISCUSSION

The bacteria isolated and identified from the surface of the different processed frozen seafood products were *B. cereus, E. aerogenes, Salmonella* sp., *Flavobacterium* sp., *Micrococcus* sp. and *S. auerus*. The same trend of bacterial growth was also reported by several workers. Fagade et al. (2005) reported the isolation of a total of 2 genera of bacteri, and 3 genera of fungi in a similar study on non-carbonated orange drink. Ikenebomeh and Elohor (2005) reported the isolation of *S. auerus, B. cereus, E. aerogenes* and other organisms in a similar study on a fresh and roasted edible worms (*Rhynchophorus phoenics*) larvae collected from 5 locations in Delta and Edo state of Nigeria.

The presence of *S. aureus* and *Salmonella* sp. was also reported in sausages sold in Abeokuta and Benin-

city, Nigeria by Oluwafemi and Simisaye (2005). According to Oluwafemi and Simisaye (2005) most of the sausage being sold as ready-to-food pose health risk to consumers, making it imperative to institute not only sanitary measures during its production and sales bit for retailers selling raw of pre-processed foods to have a steady source of power supply. Afolabi et al. (2004) reported the presence of similar microorganisms; *E. aerogenes, Salmonella* sp. and *S. aureus* in a similar study on some fruit drinks available in the market.

 0.26×10^{2}

The total counts reported in this study ranged between 1.08×10^2 to 2.86×10^4 CFU/ml. This finding is similar to the range from 1.0 to 8.0×10^4 CFU/g on Nutrient Agar as reported by of Ikenebomeh and Elohor (2005) in a similar study on the microbiological analysis of larvae and the microbial load ranging between 2.0×10^4 to 1.6×10^8 CFU/ml and coliform count between 50 to 70 cells reported by Afolabi (2005) in a similar study on hand measured rebagged powdered milk. Though the microbial counts in this study is lower than the ranges but comparable with total aerobic count ($3.5 \text{ to } 4.0 \times 10^6$ CFU/ml), *Staphylococcus* count (1.8×10^5 to 2×10^7 CFU/ml), enterobacteriaceae counts (5.09×10^8 cells) and LAB count ($1.34 \text{ to } 4.6 \times 10^8$ CFU/ml) reported in a similar study by Oluwafemi and Simisaye (2005).

Most of the organisms found on these seafood products are those commonly found in soil and water. Though the most frequent isolated index of water quality and indicators of faecal contamination; *E. coli* and *Streptococcus faecalis* were not reported in this study, the presence of other indicator organisms like *Enterobacter aerogenes* and *Salmonella* sp. might be the result of possible contamination during sales or unhygienic handling of seafood right from the processing plants. The presence of these organisms reported in this study could be an indication of faecal contamination of the water used for processing frozen seafood products or the seafood products itself and this might have adverse effect on the health of the consumers (Okonko et al., 2008).

The presence of S. auerus, a pathogenic organism of public health concern and significance in these frozen seafood products might have contaminated the processed frozen seafood products from source as a result of handling by processors. Improper handling and improper hygiene might lead to the contamination of ready-toeat food and this might eventually affects the health of the consumers (Dunn et al., 1995; Adebolu and Ifesan, 2001; Omemu and Bankole, 2005; Okonko et al., 2008). It is therefore suggested that frozen seafood processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of faecal contamination. However, the vendors/retailers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contamination of these processed frozen seafood products.

When these processed frozen seafood products are consumed raw, there is the likelihood of endangering the health of the consumers especially when the microorganisms present include pathogenic ones. From this study, it is therefore suggested that frozen seafood processing operators should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of faecal contamination. It is also preferable that these processed frozen seafood products be washed with vinegar after rinsing very well with water before dressing for consumption since vinegar can prevent bacterial growth. In no situation should the processed frozen seafood products be consumed without any form of pretreatment because they might serve as source of infection to the consumers.

REFERENCES

- Adebolu TT, Ifesan BO (2001). Bacteriological quality of vegetables used in salads. Nig. J. Microbiol. 15 (1): 81-85.
- Afolabi OR (2005). Microbial risk of hand measured rebagged powdered milk. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), UNAAB, from 6-10th November, p. 18.

- Afolabi OR, Adams RF, Popoola TOS (2004). Comparative studies and Microbial risk assessment of fruit juices and fruit drinks available in the market. In: the Book of Abstract no. 59 of the 1st International Conference on Science and National Development, organized by COLNAS, UNAAB, from 25-28th October, p. 56.
- Dunn RA, Hall WN, Altamirano JV, Dietrich SE, Robinson-Dunn B, Johnson DR. (1995). Outbreak of *Shiegella flexneri* linked to salad prepared at a central commissary in Michigan. Public Health Reports 110(5): 580-586.
- Edema MO, Omemu AM, Bankole MO (2005). Microbiological safety and quality of ready-to-eat foods in Nigeria. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, from 6-10th November, p. 26.
- Fagade OE, Ogunjobi AA, Oyelade AA (2005). Microflora of Noncarbonated orange drink. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), UNAAB, from 6-10th November, p. 16.
- Higgins C (2007). Food safety: old habits, new perspectives. In: Book and Media. J. Emerg. Infect. Dis. 13(6): 960-961.
- Ikenebomeh MJ, Elohor O (2005). Microbiology of fresh and roasted edible worms' larvae (*Rhynchophorus phoenics*). In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, from 6-10th November, p. 26.
- Jolt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST (1994). Bergey's manual of systematic bacteriology, 9th edn. Williams & Wilkins Co. Baltimore, Maryland, p. 786.
- Okonko IO, Ogunjobi AA, Kolawale OO, Adejoye OD, Ogunnusi TA (2008). Comparative studies and Microbial risk assessment of different water samples used for processing frozen sea-foods in Ijoraolopa, Lagos State, Nigeria (in press).
- Oluwafemi F, Simisaye MT (2005). Extent of microbial contamination of sausages sold in two Nigerian cities. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, from 6-10th November, p. 28.
- Omemu AM, Bankole MO (2005). Ready-to-eat (RTE) vegetable salad: effect of washing and storage temperature on the microbial quality and shel-life. In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), UNAAB, from 6-10th November, p. 28.
- Phyllis E (2007). Food safety: old habits, new perspectives. ASM Press. Herndon, Virginia, USA, p. 414.