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Microbial studies on frozen shrimps processed in Ibadan and Lagos, Nigeria

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This study reports the microbial investigation on shrimps, a frozen seafood, processed at ten different processing plants in Ibadan and Lagos, Nigeria. Samples processed and unprocessed shrimps were collected and analyzed for the presence of microorganisms. Microbial loads were enumerated using microbiological media. A total of 120 isolates characterized as Bacillus sp., Salmonella sp., Shigella sp., Enterobacter sp., Micrococcus sp., Escherichia coli, Flavobacterium sp., Staphylococcus aureus, Pseudomonas sp., Rhizopus sp., Aspergillus flavus, Aspergillus formigatus, Mucor mucido, and Saccharomyces sp. were isolated from shrimps. No Vibrio sp. was isolated from any of the shrimp sample. Rhizopus sp., A. flavis, and M. mucido were only found in unprocessed shrimp while Saccharomyces sp. and A. formigatus was found on processed shrimps only. Bacillus sp. were the most predominant species and most frequently isolated organism in this study, all the shrimps sampled haboured Bacillus sp. [20(16.7%)], followed by Salmonella sp. [18 (15.0%)], Shigella sp. [18 (15.0%)], Enterobacter sp. [13(10.8%)], Micrococcus sp. [13(10.8%)], E. coli [12(10.0%)], Flavobacterium sp. [5(4.2%)], S. aureus [5(4.2%)] and Pseudomonas sp. [1(0.8%)]. Rhizopus sp [8(6.7%)] was the most frequently implicated fungi in this study, followed by A. flavis [3(2.5%)], A. formigatus [1(0.8%)], M. mucedo [1(0.8%)], and Saccharomyces sp. [1(0.8%)]. The microbial loads at 24 and 48 h incubation showed that most of the shrimps exceed the FAO/WHO standard limit for food and water. At 24 and 48 h, processed shrimps gave total viable counts 70 - 440 and 100-1000 CFU/ml) coliform bacilli, 0 - 120 and 20 - 150 CFU/ml; Salmonella-Shigella (SS) counts, 0 - 120 and 0 - 700 CFU/ml and fungi count, 0 - 30 and 0 - 50 CFU/ml respectively, while unprocessed shrimps gave total viable counts, 390 - 1200 and 500 - 2000 CFU/ml; coliform bacilli, 120 - 350 and 380 - 1280 CFU/ml; SS counts, 80 - 1800 and 220 – 1850 CFU/ml and fungi count 70 - 260 and 120 - 600 CFU/ml respectively. The findings of this study indicates that none of the shrimps of the food processors and the water samples were within acceptable FAO/WHO/NAFDAC standards due to high colony counts, presence of indicator organisms/index of food quality - E. coli, Salmonella sp., Shigella sp., and S. aureus. This study suggests that most shrimps sold in the market may be sources of microbial food poison and intoxication hence, they are counter productive by being responsible for public health hazard and loss of revenue. Therefore, there is the need to improve on hygienic and sanitary practices in seafood processing plants and retail service outlets in order to obtain relatively safe processed seafood products for human consumption.

Key words: Microbial loads, index of food quality, microbiological quality, pathogens, shrimps.
INTRODUCTION

In Nigeria, there is a large number of public frozen seafood processing plants distributed along the country, where a considerable number of people buy their frozen seafood products daily. Serious consequences relating to national productivity and development can arise from lack of hygiene and sanitation in such outlets. The term “seafood products” or “fish” includes all fresh or saltwater finfish, molluscan shellfish, crustaceans, and other forms of aquatic animal life. Fish and shellfish are an important part of a healthful diet. They contain high quality protein and other essential nutrients which can be low in saturated fat and may contain omega-3 fatty acids. In fact, a well-balanced diet that includes a variety of fish and shellfish can contribute to heart health and children’s growth and development. But, as with any type of food, it’s important to handle seafood safely in order to reduce the risk of foodborne illness. Following these basic food safety tips for buying, preparing, and storing fish and shellfish ensures that consumers enjoy fine taste and good nutrition of seafood (FSIS, 2007; FDA, 2007a, b, c, d).

Fish products have an essential role in the traditional European diet due to their composition and the high number of fish species (SMITC, 2005). Fish products contribute significantly to “healthy diets” due to their high content on ω-3 polyunsaturated fatty acids (ω-3 PUFA), and other important components as high quality proteins, vitamins or minerals (Ackman, 1989). However, fish products are very prone to degradation (Liston, 1982). Their high water content, autochthon bacteria flora able to live at low temperatures and its high enzymatic activity, mainly autolitic, are responsible of the susceptibility of fish muscle. Among these changes, lipid oxidation is one of the most important. It leads to rancid flavours and reduces the shelf-life of fish products especially during storage (Flick and Martin, 1992). Generally, seafood products are very safe to eat. On a pound-for-pound basis, seafood is as safe as, if not safer than, other meat sources. But no food is completely safe, and problems do occur (Kurtzweil et al., 2008).

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 excretory systems in a statistically high percentage of people examined. Few of these reports however, have looked at the likely risks from a microbiological food safety point of view (Edema et al., 2005). A tender tuna steak lightly seasoned with lemon pepper and grilled over a charcoal fire is one way to please a seafood lover’s palate. Stuffed flounder, lobster thermidor, and shrimp scampi are others. But blue marlin served up with a dose of Norwalk-like virus are more likely to turn the stomach, instead of treating the palate (Kurtzweil et al., 2008). The vomiting-type outbreaks have generally been associated with rice products; however, other starchy foods such as potato, pasta and cheese products have also been implicated. Food mixtures such as sauces, puddings, soups, caseroles, pastries, and salads have frequently been incriminated in food poisoning outbreaks (FSRI, 2003; FDA, 2007a, b, c, d).

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Salmonella spp., Clostridium botulinum, Staphylococcus
aureus, Campylobacter jejuni, Yersinia enterocolitica, Yersinia pseudotuberculosis, Listeria monocytogenes, Vibrio cholerae O1, Vibrio cholerae non-O1, Vibrio parahaemolyticus and other vibrios, Vibrio vulni-ficis, Clostridium perfringens, Bacillus cereus, Aeromonas hydrophila and other spp., Plesiomonas shigelloides, Shigella spp., Miscellaneous enterics, as well as Strep-tococcus are pathogens often encountered in seafood products (FDA, 2007a,b,c,d). There is a widespread occurrence of these pathogens in animals, especially in poultry and swine. Environmental sources of the patho-gens include water, soil, insects, factory surfaces, kitchen surfaces, animal feces, raw meats, raw poultry, and raw seafoods, to name only a few (FSRI, 2003; FSIS, 2007; FDA, 2007a, b, c, d).

Pathogens such as L. monocytogenes, C. botulinum, and B. cereus are naturally present in some soil, and their presence on fresh produce is not rare. Salmonella, Escherichia coli O157:H7, Campylobacter jejuni, V. cholerae, parasites, and viruses are more likely to contaminate fresh produce through vehicles such as raw or improperly composted manure, irrigation water containing untreated sewage, or contaminated wash water. Treatment of produce with chlorinated water reduces populations of pathogenic and other micro-organisms on fresh produce but cannot eliminate them. Reduction of risk for human illness associated with raw produce can be better achieved through controlling points of potential contamina-tion in the field; during harvesting; during processing or distribution; or in retail markets, food-service facilities, or the home (CDC, 1996, 1999; PHPP, 1997; Scales et al., 2003; FDA, 2007a,b,c,d).

Various Salmonella species have long been isolated from the outside of egg shells. The present situation with S. enteritidis is complicated by the presence of the organ-ism inside the egg, in the yolk. This and other information strongly suggest vertical transmission, that is, deposition of the organism in the yolk by an infected layer hen prior to shell deposition. Foods other than eggs have also caused outbreaks of S. enteritidis disease. Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, and chocolate have been implicated in harbouring pathogenic micro-organi-sm (CDC, 1966, 1999; FDA, 2007a,b,c,d).

An outbreak of severe dysentery caused by S. dysen-teriae type 2 recently occurred at the U.S. Naval Hospital, Bethesda, Maryland. Epidemiologic investiga-tion implicated the salad bar in the active-duty staff cafeteria as the source of infection. Outbreak of Shigella flexneri at a central commissary in Michigan was linked to salad preparation. Although all Shigella spp. have been impli-cated in foodborne outbreaks at some time, Shigella sonnei is clearly the leading cause of shigellosis from food. The other species are more closely associated with contaminated water. One in particular, S. flexneri, is now thought to be in large part sexually transmitted (Dunn et al., 1995; CDC, 1996, 1999; FDA, 2007a, b, c, d).

V. parahaemolyticus bacterium is frequently isolated from the estuarine and marine environment of the United States. Both pathogenic and non-pathogenic forms of the organism can be isolated from marine and estuarine en-vvironments and from fish and shellfish dwelling in these environments. Infections with this organism have been associated with the consumption of raw, improperly cooked, or cooked, recontaminated fish and shellfish. A correlation exists between the probability of infection and warmer months of the year. Improper refrigeration of seafoods contaminated with this organism will allow its proliferation, which increases the possibility of infection. During July-August 1997, the largest reported outbreak in North America of culture-confirmed V. parahaemolyticus infections occurred. Illness in 209 persons was associated with eating raw oysters harvested from California, Oregon, and Washington in the United States and from Britain (BC) in Canada; one person died (CDC, 1998). During July-September 1998, an outbreak of V. parahaemolyticus infections associated with consumption of oysters and clams harvested from Long Island Sound occurred among residents of Connecticut, New Jersey, and New York. This is the first reported outbreak of V. parahaemolyticus linked to consumption of shellfish har vested from New York waters (CDC, 1999).

Groups A and D Streptococcus spp. can be transmitted to humans via food. Food sources include milk, ice cream, eggs, steamed lobster, ground ham, potato salad, egg salad, custard, rice pudding, and shrimp salad. In almost all cases, the foodstuffs were allowed to stand at room temperature for several hours between preparation and consumption. Entrance into the food is the result of poor hygiene, ill food handlers, or the use of un-pasteurized milk. Entrance into the food chain is due to underprocessing and/or poor and unsanitary food prepa ration (CDC, 1996, 1999; FDA, 2007a,b,c,d).

Miscellaneous enterics, Gram-negative genera include-Klebsiella, Enterobacter, Proteus, Citrobacter, Aerobacter, Providencia, Serratia and E. coli are organisms which may be recovered from natural environ-ments such as forests and freshwater as well as from farm produce (vegetables) where they reside as normal microflora. These bacteria have been recovered from dairy products, raw shellfish, and fresh raw vegetables. The organisms occur in soils used for crop production and shellfish har vesting waters and, therefore, may pose a health hazard (Frampton and Restaino, 1993; CDC, 1996, 1999; FDA, 2007a, b, c, d).

In 1995, the national Centers for Disease Control and Prevention reported 34 incidences of food poisoning in people who had eaten oysters harvested from certain southern U.S. waters. Health experts blamed the flu-like illness on a virus similar to the Norwalk virus, which is usually introduced into fishing areas by human sewage. In 1997, 26 employees of the World Bank headquarters in Washington, D.C., developed headaches, dizziness, nausea, and rashes several hours after eating blue marlin.
served in their workplace cafeteria. An emergency room doctor who treated some of the victims attributed the illness to scombroid poisoning, which is caused by a toxin produced when certain fish product spoil (Kurtzweil et al., 2008).

FDA (2007a, b, c, d) recommends a continuous prioritization of all manufacturers of high risk fishery products, particularly processors of scombroid species and cooked ready-to-eat products, for annual inspection as well as processors and importers of aquaculture products to undergo increased inspection and training. The potential of seafood and water to harbour microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Younes and Bartram, 2001; Wright et al., 2004). Water-related diseases continue to be one of the major health problems globally. It is estimated that 80% of all illnesses are linked to use of food and water of poor micro-biological quality (WHO, 2005). Treatment of farm produce such as seafood with chlorinated water reduces populations of pathogenic and other microorganisms on fresh produce but cannot eliminate them. Reduction of risk for human illness associated with raw produce can be better achieved through controlling points of potential contamination in the field, during harvesting, during processing or distribution, or in retail markets, food-service facilities, or the home (USDA, 2007; FDA, 2007a,b,c,d; FAO/WHO, 2007).

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). A good knowledge of the microbial qualities of raw frozen seafood is necessary so as to guide its suitability for consumption. Thus, regular microbiological analysis of seafood products at source or processing plants must be carried out to determine or check the effectiveness of processing and packaging. This study sought to evaluate the microbial quality on frozen shrimps processed in Ibadan and Lagos, Nigeria. The microbial loads of processed and unprocessed shrimps samples collected from 10 different seafood processing plants is shown in Table 1 and 2. Samples AP to JP are processed shrimp samples while samples UA to UJ are unprocessed shrimp samples collected from 10 different processing plants. The microbial load at 24 h incubation differs from one processing plants to the other (Table 1). The total viable count (TVC) ranges from 70 to 440 CFU/ml for the processed shrimp samples and 390 to 1200 CFU/ml for unprocessed shrimps samples. These exceed the FAO/WHO standard limit 1.0 x 10^2 CFU/ml for food products and water (Table 1). Table 1 also indicates that of all the processed shrimp samples, sample FP had the highest total viable count of 440 CFU/ml, followed by sample GP (260 CFU/ml), HP (150 CFU/ml), EP (130 CFU/ml), IP (120 CFU/ml) and 80 CFU/ml for sample AP and BP respectively and sample JP had the least count (70 CFU/ml) while of all the unprocessed shrimps; sample UE had the highest total viable count of 1200 CFU/ml, followed by UH (1160 CFU/ml), UA (640 CFU/ml), UI (580 CFU/ml) and UC and UF with 560 CFU/ml each (Table 1).

The probable numbers of total coliform bacilli ranges from zero to 120 MPN/ml for of 1 ml processed shrimps samples and 120 to 350 MPN/ml for unprocessed shrimp samples, this also exceeds the FAO/WHO standard limit of zero MPN/ml for food products and water (Table 1). Of all processed samples, sample HP had the highest coliform count (120 MPN/ml), followed by samples DP and FP having 40 MPN/ml each and sample BP had zero MPN/ml while among the unprocessed shrimps; sample UA had the highest coliform count 350 MPN/ml, followed by UB and UH having 330 MPN/ml each. All the unprocessed samples had a very high MPN values, highly exceeding the FAO/WHO standard limit for food products (Table 1).

The Salmonella-Shigella count (SSC) ranges from zero CFU/ml to 120 CFU/ml for processed shrimp samples and 80 to 1800 CFU/ml for unprocessed shrimp samples (Table 1). After 24 h incubation, samples of processed shrimps AP and BP had no growth of Salmonella and Shigella sp. Samples JP had the highest Salmonella and Shigella count with 400 CFU/ml, followed by samples EP and DP having 240 and 230 CFU/ml respectively (Table 1), while unprocessed shrimps sample had heavy loads of Salmonella and Shigella ranging from 80 to 1800 CFU/ml.

Sample UH had the highest Salmonella and Shigella count with 400 CFU/ml, followed by samples EP and DP having 240 and 230 CFU/ml respectively (Table 1), while unprocessed shrimps sample had heavy loads of Salmonella and Shigella ranging from 80 to 1800 CFU/ml.
Table 1. Microbial loads of processed and unprocessed shrimp samples after 24 h incubation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TVC (CFU/ml)</th>
<th>TCC (MPN/ml)</th>
<th>SSC (CFU/ml)</th>
<th>FC (CFU/ml)</th>
<th>Identified organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Processed shrimps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>80</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>Bacillus sp., Enterobacter sp.</td>
</tr>
<tr>
<td>BP</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Bacillus sp., E. coli</td>
</tr>
<tr>
<td>CP</td>
<td>110</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>Bacillus sp., E. coli, Favorbacterium sp., Micrococcus sp, Salmonella sp., Shigella sp.</td>
</tr>
<tr>
<td>DP</td>
<td>100</td>
<td>40</td>
<td>230</td>
<td>0</td>
<td>Bacillus sp., Enterobacter sp.</td>
</tr>
<tr>
<td>EP</td>
<td>130</td>
<td>10</td>
<td>240</td>
<td>0</td>
<td>Pseudomonas sp., Salmonella sp., Shigella sp.</td>
</tr>
<tr>
<td>FP</td>
<td>440</td>
<td>40</td>
<td>80</td>
<td>30</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Favorbacterium sp., Micrococcus sp, Salmonella sp., Shigella sp., Staphylococcus sp.</td>
</tr>
<tr>
<td>GP</td>
<td>260</td>
<td>20</td>
<td>100</td>
<td>0</td>
<td>A. formigatus, Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Salmonella sp., Shigella sp.</td>
</tr>
<tr>
<td>HP</td>
<td>150</td>
<td>120</td>
<td>180</td>
<td>20</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Shigella sp., Staphylococcus sp.</td>
</tr>
<tr>
<td>IP</td>
<td>120</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Shigella sp., Favorbacterium sp., Micrococcus sp, Salmonella sp.</td>
</tr>
<tr>
<td>JP</td>
<td>70</td>
<td>10</td>
<td>400</td>
<td>0</td>
<td>Bacillus sp., Enterobacter sp., Micrococcus sp, Salmonella sp., Shigella sp., Staphylococcus sp</td>
</tr>
<tr>
<td><strong>Unprocessed shrimps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>640</td>
<td>350</td>
<td>100</td>
<td>70</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Salmonella sp., Shigella sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UB</td>
<td>390</td>
<td>330</td>
<td>80</td>
<td>100</td>
<td>Bacillus sp., Micrococcus sp, M. mucido, Salmonella sp., Shigella sp.</td>
</tr>
<tr>
<td>UC</td>
<td>560</td>
<td>190</td>
<td>200</td>
<td>80</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Flavobacterium sp, Shigella sp, Salmonella sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UD</td>
<td>460</td>
<td>250</td>
<td>480</td>
<td>120</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Shigella sp, Salmonella sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UE</td>
<td>1200</td>
<td>210</td>
<td>360</td>
<td>90</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Shigella sp, Salmonella sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UF</td>
<td>560</td>
<td>150</td>
<td>165</td>
<td>150</td>
<td>Bacillus sp., Enterobacter sp., Micrococcus sp, Shigella sp, Salmonella sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UG</td>
<td>390</td>
<td>250</td>
<td>100</td>
<td>130</td>
<td>Bacillus sp., Enterobacter sp., Shigella sp, Salmonella sp., Staphylococcus sp, Rhizopus sp.</td>
</tr>
<tr>
<td>UH</td>
<td>1160</td>
<td>330</td>
<td>1800</td>
<td>70</td>
<td>Bacillus sp., Enterobacter sp., Flavobacterium sp, Shigella sp, Salmonella sp., Staphylococcus sp, A. flavis</td>
</tr>
<tr>
<td>UI</td>
<td>580</td>
<td>120</td>
<td>100</td>
<td>200</td>
<td>Bacillus sp., E. coli, Shigella sp, Salmonella sp., Staphylococcus sp, Rhizopus sp.</td>
</tr>
<tr>
<td>UJ</td>
<td>460</td>
<td>240</td>
<td>620</td>
<td>260</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Shigella sp, Salmonella sp., Rhizopus sp, A. flavis</td>
</tr>
</tbody>
</table>

TVC = Total Viable Count; TCC = Total Coliform Count; SSC = Salmonella-Shigella Count; FC = Fungi Count; CFU = Colony forming unit; ML = Million litre; AP-JP = Processed shrimp samples; UA-UJ = Unprocessed shrimp samples.

The fungi count (FC) for the samples ranged from zero CFU/ml to 30 CFU/ml for processed shrimp samples and 70 to 260 CFU/ml for unprocessed shrimp samples (Table 1). Only two of processed shrimps had no fungi growth after 24 h incubation, samples HP and FP having 20 and 30 CFU/ml respectively while all the unprocessed shrimps had fungi growth with sample UJ having the highest fungi count 260 CFU/ml, followed by samples UI, UF, UG and UD having 200, 150, 130 and 120 CFU/ml respectively (Table 1).

Table 2 shows the microbial loads of processed and unprocessed shrimp samples collected from different frozen seafood processing plants incubated for 48 h. Total viable count at 48 h of incubation were very different from one shrimp sample to the other and this ranged from 100 to 1000 CFU/ml for processed shrimps.
Table 2. Microbial loads of processed and unprocessed shrimp samples after 48 h incubation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TVC (CFU/ml)</th>
<th>TCC (MPN/ml)</th>
<th>SSC (CFU/ml)</th>
<th>FC (CFU/ml)</th>
<th>Identified organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Processed shrimps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>140</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>Bacillus sp., Enterobacter sp.</td>
</tr>
<tr>
<td>BP</td>
<td>100</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>Bacillus sp., E. coli</td>
</tr>
<tr>
<td>CP</td>
<td>350</td>
<td>40</td>
<td>50</td>
<td>0</td>
<td>Bacillus sp., E. coli, Favobacterium sp., Micrococcus sp., Salmonellla sp., Shigella sp.</td>
</tr>
<tr>
<td>DP</td>
<td>160</td>
<td>70</td>
<td>420</td>
<td>0</td>
<td>Bacillus sp., Enterobacter sp. Micrococcus sp, Pseudomonas sp., Salmonellla sp., Shigella sp.</td>
</tr>
<tr>
<td>EP</td>
<td>500</td>
<td>20</td>
<td>420</td>
<td>0</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Shigella sp., Micrococcus sp, Salmonellla sp., Shigella sp., Sacchromyces sp.</td>
</tr>
<tr>
<td>FP</td>
<td>1000</td>
<td>50</td>
<td>110</td>
<td>50</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Favobacterium sp., Micrococcus sp, Salmonellla sp., Shigella sp.</td>
</tr>
<tr>
<td>GP</td>
<td>600</td>
<td>30</td>
<td>140</td>
<td>0</td>
<td>Bacillus sp., Micrococcus sp, Salmonellla sp., Shigella sp., S. auerus</td>
</tr>
<tr>
<td>HP</td>
<td>800</td>
<td>150</td>
<td>300</td>
<td>50</td>
<td>A. formigatus, Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Salmonellla sp., Shigella sp.</td>
</tr>
<tr>
<td>IP</td>
<td>500</td>
<td>40</td>
<td>50</td>
<td>0</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Shigella sp., Favobacterium sp., Micrococcus sp, Salmonellla sp.</td>
</tr>
<tr>
<td>JP</td>
<td>140</td>
<td>60</td>
<td>700</td>
<td>0</td>
<td>Bacillus sp., Enterobacter sp., Micrococcus sp, Salmonellla sp., Shigella sp., S. auerus</td>
</tr>
<tr>
<td><strong>Unprocessed shrimps</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>1000</td>
<td>800</td>
<td>500</td>
<td>120</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Salmonellla sp., Shigella sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UB</td>
<td>500</td>
<td>600</td>
<td>300</td>
<td>180</td>
<td>Bacillus sp., Micrococcus sp, Mucor mucido, Salmonellla sp., Shigella sp.</td>
</tr>
<tr>
<td>UC</td>
<td>600</td>
<td>900</td>
<td>350</td>
<td>120</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Flavobacterium sp., Shigella sp., Salmonellla sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UD</td>
<td>800</td>
<td>1200</td>
<td>530</td>
<td>180</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Shigella sp., Salmonellla sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UE</td>
<td>1600</td>
<td>980</td>
<td>470</td>
<td>125</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Shigella sp., Salmonellla sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UF</td>
<td>1000</td>
<td>800</td>
<td>250</td>
<td>220</td>
<td>Bacillus sp., Enterobacter sp., Micrococcus sp, Shigella sp., Salmonellla sp., Rhizopus sp.</td>
</tr>
<tr>
<td>UG</td>
<td>690</td>
<td>380</td>
<td>220</td>
<td>300</td>
<td>Bacillus sp., Enterobacter sp., Shigella sp, Salmonellla sp., S. auerus., Rhizopus sp.</td>
</tr>
<tr>
<td>UH</td>
<td>2000</td>
<td>440</td>
<td>1850</td>
<td>130</td>
<td>Bacillus sp., Enterobacter sp., Flavobacterium sp., Shigella sp, Salmonellla sp., S. auerus, A. flavis</td>
</tr>
<tr>
<td>UI</td>
<td>900</td>
<td>740</td>
<td>240</td>
<td>450</td>
<td>Bacillus sp., E. coli, Shigella sp, Salmonellla sp., S. auerus, Rhizopus sp.</td>
</tr>
<tr>
<td>UJ</td>
<td>600</td>
<td>1280</td>
<td>860</td>
<td>600</td>
<td>Bacillus sp., E. coli, Enterobacter sp., Micrococcus sp, Shigella sp, Salmonellla sp., Rhizopus sp., A. flavis</td>
</tr>
</tbody>
</table>

TVC = Total Viable Count; TCC = Total Coliform Count; SSC = Salmonella-Shigella Count; FC = Fungi Count; CFU = Colony forming unit; MI = Millilitre; AP-JP = Processed shrimp samples; UA-UJ = Unprocessed shrimp samples.

and 500 to 2000 CFU/ml for unprocessed shrimps. Sample FP also had the highest total viable count of all the processed shrimps (1000 CFU/ml), followed by samples HP, GP, EP and IP having 800, 600, 500 and 500 CFU/ml, respectively, while of all the unprocessed shrimps; UH had the highest total viable count having 2000 CFU/ml, followed by samples UE, UA and UG having 1600, 1000 and 1000 CFU/ml respectively (Table 2). At 48 h incubation microbial load of all the samples exceed the FAO/WHO standard limit 100 CFU/ml (Table 2).

Also, the probable numbers of coliform bacilli ranged from 20 to 150 CFU/ml and 380 to 1280 CFU/ml for processed and unprocessed shrimp respectively (Table 2), indicating that unprocessed shrimps UJ had the highest coliform count with 1280 MPN/ml, followed by UD, UE, UC, UA, and UF having 1200, 980, 900, 800 and 800 MPN/ml respectively. This indicates that all unprocessed
Table 3. Frequency of occurrence of microorganisms isolated from processed and unprocessed shrimp samples.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. (%)</th>
<th>Processed shrimps (%)</th>
<th>Unprocessed shrimps (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP</td>
<td>BP</td>
<td>CP</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>20 (16.7)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E. coli</td>
<td>12 (10.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>13 (10.8)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Favobacterium sp.</td>
<td>5 (4.2)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>13 (10.8)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>2 (1.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>18 (15.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>18 (15.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. auerus.</td>
<td>5 (4.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. formigatus,</td>
<td>1 (0.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. flavis</td>
<td>3 (2.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>8 (6.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. mucido</td>
<td>1 (0.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sacchromyces sp.</td>
<td>1 (0.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Overall Total</td>
<td>120 (100)</td>
<td>52 (43.3)</td>
<td></td>
</tr>
</tbody>
</table>

Key:
AP-JP = Processed shrimp samples; UA-UJ = Unprocessed shrimp samples.

shrimps exceeds FAO/WHO standard limit of 0 MPN/ml (Table 2).

The Salmonella-Shigella count after 48 h incubation showed a high count for all the shrimp samples ranging from zero to 700 CFU/ml for processed shrimps and 220 to 1850 CFU/ml for unprocessed shrimps (Table 2).

After 48 h incubation, fungi count of the unprocessed shrimp samples ranges from 120 to 600 CFU/ml. UJ had the highest fungi count 600 CFU/ml, followed by samples UI, UG and UF having 450, 300, and 220 CFU/ml respectively (Table 2).

A total of 120 isolates were obtained and identified as Bacteria, fungi and yeast. The bacteria isolates include *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Flavobacterium* sp., *Micrococcus* sp., *Pseudomonas* sp., *Salmonella* sp., *Shigella* sp., *S. auerus*. No *Vibrio* sp. was isolated on TCBS agar. The fungi isolates were identified as *A. formigatus*, *A. flavis*, *M. mucido*, *Rhizopus* sp., and *Sacchromyces* sp.

Table 3 shows the frequency of the organisms isolated from the processed and unprocessed shrimp samples. The highest number of the isolates was obtained from unprocessed shrimp samples which constituted 68 and 52% of the isolates were obtained from of processed shrimp samples (Table 3).

*Bacillus* sp. [20 (16.7%)] were the most frequently isolated organism from all the shrimps samples, followed by *Salmonella* sp. [18 (15.0%)], *Shigella* sp. [18 (15.0%)], *Enterobacter* sp. [13 (10.8%)], *Micrococcus* sp. [13 (10.8%)], *Escherichia coli* [12 (10.0%)], *Flavobacterium* sp. [5 (4.2%)], *S. auerus* [5(4.2%)] and *Pseudomonas* sp. [1 (0.8%)] (Table 3). *Rhizopus* sp [8 (6.7%)] was the most frequently fungi isolates implicated in this study, followed by *A. flavis* [3 (2.5%)], *A. formigatus* [1 (0.8%)], *M. mucido* [1 (0.8%)], and *Sacchromyces* sp. [1 (0.8%)] (Table 3).

These pathogens were present in the processed and unprocessed shrimps collected from frozen seafood processing plants. *Bacillus* sp. was isolated from all the shrimp samples while *Enterobacter* sp. was isolated from 5 processed shrimps and 8 unprocessed shrimp samples and *Escherichia coli* were isolated from both processed and
unprocessed shrimp samples in equal magnitude, 6 samples each (Table 3). Staphylococcus sp. was only isolated from 2 processed and 3 unprocessed shrimp samples. Sacchromyces sp. and A. formigatus was isolated from processed shrimp samples only and all other fungi isolates- Rhizopus sp., A. flavis, and M. mucido were only found in unprocessed shrimp samples (Table 3). No Vibrio sp. was isolated from any of the shrimp sample.

DISCUSSION

In this present study, all shrimp samples haboured Bacillus sp. while prominent microorganisms variously haboured include Salmonella sp., Shigella sp., Enterobacter sp., Micrococcus sp., E. coli, Rhizopus sp., Flavobacterium sp., Staphylococcus sp., A. flavis, Pseudomonas sp., A. formigatus, M. mucido, and Saccharomyces sp. No Vibrio sp. was isolated from any of the shrimp sample. Shrimps may be sources of microbial food poison and food intoxication hence, shrimps may be counter productive by being responsible for public health hazard and loss of revenue. All these pathogen isolated in this study are of food processing and public health implication and hence, hazardous and injurious to human health if consumed.

Most of the organisms found on these shrimps are those commonly found in soil and water. But an organism like E. coli isolated from some of the shrimps is an indication of faecal contamination of the shrimps and might affect the health of the consumer. S. aureus, another organism found on the shrimps is also a pathogenic organism of public health significance. This organism might have contaminated the shrimps as a result of handling. This is also in accordance to the assertion of Dunn et al. (1995) and Omemu and Bankole (2005) that improper handling and improper hygiene might lead to the contamination of ready-to-eat food such as vegetable salad and this might eventually affects the health of the consumers.

The pathogens isolated in this present study are similar to the microorganisms reported by Olalwe et al. (2005), nine bacterial genera and two fungi in a similar study which include S. aureus, E. aerogenes, Streptococcus faecalis, E. coli, among other organisms. The presence of Bacillus sp., Salmonella sp., Shigella sp., Enterobacter sp., Micrococcus sp., E. coli, Rhizopus sp., Flavobacterium sp., Staphylococcus sp., A. flavis, Pseudomonas sp., A. formigatus, M. mucido, and Saccharomyces sp. reported in this study is also in agreement with the findings of Adesokan et al. (2005) who reported the presence of Bacillus sp. and E. coli among other organisms and Bankole et al. (2004, 2005) who reported the presence of S. aureus, S. epidermidis, Bacillus sp. E. coli, Enterobacter sp., Pseudomonas sp., Shigella sp., Saccharomyces sp., Rhizopus sp., and Aspergillus sp. The presence of A. flavus, Saccharomyces sp., Bacillus sp. and Micrococcus sp. in this shrimps is also comparable with the findings of Fagade et al. (2005) in a study on the microbiological qualities of some non-carbonated orange drinks who reported the same organisms as being present in contamination of the drinks.

The most frequently isolated index of water/food quality and indicators of faecal contamination such as E. coli were isolated in this present study and the presence of some indicator and other organisms in this study is of special concern and perhaps the greatest danger associated with shrimps is contamination by human excrement (Apantaku et al., 1998; Edema et al., 2001; Okonko et al., 2008).

Most of the organisms found in this study are those commonly found in soil and water. But the presence of other indicator organisms like E. coli, S. faecalis, Salmonella sp., Shigella sp., and E. aerogenes in those water samples might be the result of possible contamination during sales or unhygienic handling of seafood right from the processing plants. The presence of the most frequently isolated index of water quality and indicators of faecal contamination such as E. coli and S. faecalis, reported in this study is an indication of faecal contamination of the water used for processing frozen seafood products as a result of possible burst along pipe lines or unhygienic handling of the water right from the treatment plant for tap water and borehole water (Edema et al., 2001; Okonko et al., 2008a, b) or contamination of the seafood products itself during processing or directly from source and this might have adverse effect on the health of the consumers (Adebolu and Ifesan, 2001; Okonko et al., 2008a, b).

The presence of S. aureus, Salmonella sp. and Shigella sp.- pathogenic organisms of public health concern and significance in these frozen seafood products and these 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-to-eat foods and this might eventually affects the health of the consumers (Dunn et al., 1995; Adebolu and Ifesan, 2001; Bankole et al., 2004; Afolabi, 2005; Omemu and Bankole, 2005; Okonko et al., 2008c). 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 processors/handlers/sellers 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.

The isolation of Salmonella sp. and Shigella sp. in almost all the shrimp samples also confirmed the much acclaimed information that various Salmonella species have long been isolated from the outside of egg shells and raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings,
dried gelatin, peanut butter, cocoa, and chocolate have been implicated in harbouring pathogenic microorganisms. The finding of this study and other information strongly suggest foods other than eggs have also caused outbreaks of *S. enteritidis* disease (CDC, 1966, 1999; FDA, 2007a,b,c,d). Also, *Shigella* sp. is frequently found in salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, milk and dairy products, and poultry. Contamination of these foods is usually through the fecal-oral route. Faecally contaminated water and unsanitary handling by food handlers are the most common causes of contamination (Dunn et al., 1995; CDC, 1996, 1999; FDA, 2007a, b, c, d).

The findings of this present study confirms that a wide variety of foods including meats, milk, vegetables, and fish have been associated with the diarrheal type food poisoning resulting from *Bacillus* spp. The vomiting-type outbreaks have generally been associated with rice products; however, other starchy foods such as potato, pasta and cheese products have also been implicated. Food mixtures such as sauces, puddings, soups, casseroles, pastries, and salads have frequently been incriminated in food poisoning outbreaks (FSRI, 2003; FDA, 2007a, b, c, d).

The microbial studies on shrimps suggests that there is need to improve on hygienic and sanitary practices in public frozen seafood processing outlets in order to obtain relatively safe products for consumption. The presence of *S. aureus* and *Salmonella* sp. was also reported in sausages sold in Abeokuta and Benin-city, Nigeria in a similar study 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 but for retailers selling raw of pre-processed foods to have a steady source of power supply.

The presence of indicator and other organisms examined in this study is of special concern and perhaps the greatest danger associated with shrimps used for food preparation, eating purposes and for other human consumption is contamination by human excrement (Edema et al., 2001; Okonko et al., 2008a, b). The need for microbial assessment of shrimps and other seafood products processed and re-packaged for human consumption to reduce possible contamination (Afolabi et al., 2004; Fagade et al., 2005). From the findings of this study, it is therefore necessary to recommend that owners of seafood processing plants and food processors/handlers/sellers should be educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation. Shrimps to be used for consumption purposes should be adequately cooked before use in preparing food for human consumption and NAFDAC should ensure and enforce strict compliance of the recommended seafood standards as regards the production and sales of processed and re-packaged seafood products such as shrimps.

**REFERENCES**


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