Comparative studies and microbial risk assessment of different water samples used for processing frozen sea- foods in Ijora-olopa, Lagos State, Nigeria

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Comparative studies and microbial risk assessment of different water samples used for processing frozen sea-foods in Ijora-olopa, Lagos State, Nigeria

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This study reports the comparative studies and microbial risk assessment of different water samples used for processing frozen sea-foods in Ijora-olopa, Lagos State, Nigeria. Twelve different samples of well, tap, borne-hole, river water used for processing frozen sea-foods were collected from different processing shops in Ijora-olopa, Lagos. These water samples were microbiologically analyzed for the presence of microorganisms. Total plate counts and total coliform counts were enumerated using Plate Count Agar (PCA) and Eosin Methylene Blue (EMB) Agar, respectively. The total count of most the water samples ranges between 0.48×10^2 and 2.04×10^2 CFU/ml, exceeding the limit of 1.0×10^2 CFU/ml. The coliform counts per 100 ml of water samples ranged between 15 and 184 cells. Fifteen (15) isolates were characterized from the samples on PCA with percentage of occurrence of different microorganisms characterized as follows: *Bacillus cereus* (33.3%), *Enterobacter aerogenes* (33.3%), *Flavobacterium* sp. (13.3%), *Micrococcus* sp. (6.7%), *Pseudomonas aerugionosa* (6.7%) and *Staphylococcus auerus* (6.7%). Some of these organisms are of public health significance. Studies show the possibilities of survival of pathogenic microorganisms during water treatment/seafood processing. Therefore, stringent quality assurance measures should be put in order to safe guard the health of the consumers.

Key words: Coliform count, microorganisms, microbiological quality, quality assurance, processing, seafood, total count.

INTRODUCTION

In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system (Okonko et al., 2008). Increase in human population has exerted an enormous pressure on the provision of safe drinking water especial-

ly in areas of developing countries (Umeh et al., 2005). Unsafe water is a global public health threat, placing persons at risk for a host of diarrheal and other diseases as well as chemical intoxication (Hughes and Koplan, 2005). Unsanitary water has particularly devastating effects on young children in the developing world. Each year, >2 million persons, mostly children <5 years of age, die of diarrheal disease (Kosek et al., 2003; Parashar et al., 2003). For children in this age group, diarrheal disease accounted for 17% of all death from 2000 to 2003

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(WHO, 2005), ranking third among causes of death, after neonatal causes and acute respiratory infections.

Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate water supplies and sanitation (WHO, 2004) conditions affecting a large part of the world's population (Hughes and Koplan, 2005). An estimated 1.1 billion persons (one sixth of the world's population) lack access to clean water and 2.6 billion to adequate sanitation (WHO, 2005; Hughes and Koplan, 2005).

The principal objectives of municipal water are the production and the distribution of safe water that is fit for human consumption (Lamikanra, 1999; Okonko et al., 2008). Recently in Nigeria, drinking water is commercially available in easy-to-open 50 – 60 ml polyethylene sacks known as satchet/pure water (Umeh et al., 2005). Conformation with microbiological standard is of special interest because of the capacity of water to spread diseases within a large population. Although the standards vary from place to place, the objective anywhere is to reduce the possibility of spreading water-borne disease in addition to being pleasant to drink, which implies that it must be wholesome and palatable in all respects (Edema et al., 2001; Okonko et al., 2008).

A collaborative, interdisciplinary effort to ensure global access to safe water, basic sanitation, and improved hygiene is the foundation for ending cycle of poverty and diseases (Hughes and Koplan, 2005). At the end of 2000 United Nations (UN) Millennium Summit, member states adopted a set of 8 goals and related targets and indicators aimed at helping end human poverty and its ramifications (Sachs and McArthur, 2005).

According to Hughes and Koplan (2005), among these millennium Development Goals is a call to halve by the year 2015 the proportion of persons without sustainable access to safe drinking water and basic sanitation. Towards the end of March 2005, the UN launched the "International Decade for Action: Water for Life 2005 - 2015" (UN, 2005; Bartram et al., 2005). Success in reaching these targets will help achieve the other goals, increase work force productivity, and substantially reduce the amount of time that women and children spend collecting and storing water, which will free them to pursue other productive and educational activities (Hughes and Koplan, 2005).

According to Bartram et al. (2005), the WHO-sponsored International Network for the Promotion of Safe Household Water Treatment and Storage, a global collaboration of UN and bilateral agencies, non-governmental organizations, research institutions, and the private sector, could serve as a model for improving coordination of international efforts in this area of global safe water, sanitation, and hygiene.

Innovative approaches toward improving water, sanitation, and hygiene must be implemented and evaluated. A number of studies conducted in a variety of geographic settings have shown that interventions such as point-of-use disinfection of water and educational efforts to improve personal hygiene help reduce disease prevalence (Clasen and Cairncross, 2004). These studies also highlighted the importance of tailoring such interventions to local situations (Hughes and Koplan, 2005).

A recent study in an area in rural western Kenya that had turbid source of water found that household use of flocculants disinfectant preparation helped to reduce the prevalence of diarrhea in children <2 years of age (Crump et al., 2005). Studies in refugee camps in Africa (Peterson et al., 1998) and urban slums in Asia (Luby et al., 2005) have reported that hand-washing with soap reduced the prevalence of diarrhea in all age groups (Peterson et al., 1998) and lowered the incidence of diarrhea and pneumonia in children <5 years of age (Luby et al., 2005).

The reduced incidence of pneumonia reported by Luby et al. (2005) is noteworthy and warrants further study. Although interventions for improving sanitation have lagged behind those for water, promising advances have been reported, especially in the development of ecologic sanitation systems (Hughes and Koplan, 2005). A good knowledge of the bacteriological and chemical qualities of raw water is necessary so as to guide its suitability for use. Thus, regular physico-chemical and bacteriological analysis of water at source must be carried out to determine or check the effectiveness of treatment process (Okonko et al., 2008).

This current study reports on the comparative microbial risk assessment of different water samples used for processing frozen sea-foods in Ijora-olopa, Lagos State, Nigeria

MATERIALS AND METHOD

Samples of borne-hole tank, tap, well, and river water were collected at different locations in Ijora-olopa, Lagos state. Water representing different turbidities were collected in sterile two water plastic containers and were taken to the laboratory and analyzed within 6 h (maximum transit time – 4 h, maximum process time - 2 h). NAFDAC approved Eva Table water (produced by Nigeria Bottling Company, Coca-Cola) was used as control. Samples A, B, C and E are water samples from a borehole water supply. Sample D is a NAFDAC approved sachet water. Samples F, G, H, and J are well water samples. Samples I and K are river water samples. Water samples were analyzed for physiochemical and bacteriological quality and the chemical characteristics were determined by the methods of FAO (1997a); Fawole and Oso (2001) and Okonko et al. (2008). Temperature was measured at the point of collection using a digitron thermometer (model 275-K) as described by the methods of FAO (1997b) and Okonko et al. (2008) and standardized mercury in glass centigrade thermometer as described by Edema et al. (2001) and Okonko et al. (2008).

The media used for the bacteriological analysis of water include plate count agar (PCA), nutrient agar (NA), lactose broth (LB), and Eosin Methylene blue agar (EMB). All the media used were weighed out and prepared according to the manufacture's specification, with respect to the given instructions and directions. A serial dilution method was used for total viable count and the presumptive test for coliforms. The water samples were then inoculated separately on different Plate Count Agar (PCA), Eosin Methylene Blue (EMB) Agar and Potato Dextrose Agar (PDA) plates and the plates were

Table 1. Physico-chemical properties of the water samples.

Sample	Colour	Odour	Taste	Presence of particles	pH	Temp (°C)
A	Colourless	Odourless	Tasteless	None	6.6	27.5
B	Colourless	Odourless	Tasteless	None	6.8	25.5
C	Colourless	Odourless	Tasteless	None	7.2	26.0
D	Colourless	Odourless	Tasteless	None	7.5	26.5
E	Colourless	Odourless	Tasteless	None	7.4	25.0
F	Slightly turbid	Odourless	Offensive	Suspended solids	6.8	25.2
G	Colourless	Odourless	Tasteless	None	6.8	27.0
H	Colourless	Odourless	Tasteless	None	6.7	26.3
I	Slightly turbid	Odourless	Offensive	Few particles	6.7	25.2
J	Colourless	Odourless	Tasteless	None	6.5	27.0
K	Slightly turbid	Odourless	Tasteless	Few particles	7.5	26.4
Standard limit	Colourless	Not offensive	Not offensive	No visible solids	6.5-8.5	

Samples A, B, C and E are water samples from a borehole water supply. Sample D is a NAFDAC approved sachet water. Samples F, G, H, and J are well water samples. Samples I and K are river water samples.

Table 2. Total counts and most probable number (MPN) of coliform/100 ml of water samples.

Sample	Total count (CFU/ml)	MPN/100 ml
A	2.04×10^2	184
B	0.87×10^2	15
C	1.96×10^2	27
D	Nil	Nil
E	0.97×10^2	64
F	Nil	Nil
G	Nil	Nil
H	Nil	Nil
I	Nil	Nil
J	Nil	Nil
K	0.48×10^2	15
Standard limit	1.0×10^2	Nil

Samples A, B, C and E are water samples from a borehole water supply. Sample D is a NAFDAC approved sachet water. Samples F, G, H, and J are well water samples. Samples I and K are river water samples.

incubated at 37°C for 24 - 48 h for evidence of growth. Pure isolates of resulting growth were identified using morphological and biochemical methods as described by Jolt et al. (1994). The sterility of each batch of test medium was confirmed by incubating one or two uninoculated tubes or plates along with the inoculated tests. The uninoculated tubes or plates were always examined to show no evidence of bacterial growth.

RESULTS

The physico-chemical properties of the water samples are shown in Table 1. Some of the potable water samples, particularly the bore-hole tank, tap, well, and the river water samples did not comply with the standard limits for drinking water and waste discharges.

All the water samples were colourless except a deviation sample F, I and K that are slightly turbid. All samples were odourless. All samples were tasteless except F and I that are offensive. The pH ranged of 6.5 to 7.5 while temperature ranged from 25 to 27.5°C (Table 1). The pH as recorded for the water samples and the pH for tap water and borehole water could be considered as being within acceptable range for natural waters. As far as the pH is concerned they vary from pH range of 6.5 – 7.5 indicating that sample D and K had the highest pH value of 7.5.

The microbiological analysis of the water and waste water samples is shown in Table 2. The total count of most the water samples ranges between 0.48×10^2 and 2.04×10^2 CFU/ml, exceeding the limit of 1.0×10^2 CFU/ml (Table 2). The total count for sample A and C were generally high exceeding the limit of 1.0×10^2 CFU/ml (Table 2). The coliform counts per 100 ml of water samples ranged between 15 and 184 cells (Table 2), also exceeding the standard limit. It indicates that water sample A stream had the highest coliform counts of 184 cells followed by water sample E having 64 cells while water sample D, F, G, H, I, and J had no coliform growth.

The bacteria isolated from the water samples are shown in Table 3. These identified isolates include *Bacillus cereus* [5 (33.3%)], *Enterobacter aerogenes* [5 (33.3%)], *Flavobacterium* sp. [2 (13.3%)], *Micrococcus* sp. [1 (6.7%)], *Pseudomonas aerugionosa* [1 (6.7%)] and *Staphylococcus auerus* [1 (6.7%)] (Table 3). *Bacillus* sp., and *E. aerogenes* were most frequently isolated being present in most of the water samples while *Flavobacterium* sp. was only isolated from water sample A and E. *Micrococcus* sp. and *P. aerugionosa* was only isolated from water sample C while *S. aureus* was only isolated from water sample A. No organism was isolated in water

Table 3. Bacterial isolates from water samples.

Bacterial Isolates	Frequency No. (%)	Water samples										
		A	B	C	D	E	F	G	H	I	J	K
Bacillus cereus	5 (33.3)	+	+	+	-	+	-	-	-	-	-	+
Enterobacter aerogenes	5 (33.3)	+	+	+	-	+	-	-	-	-	-	+
Flavobacterium sp.	2 (13.3)	+	-	-	-	+	-	-	-	-	-	-
Micrococcus sp.	1 (6.7)	-	-	+	-	-	-	-	-	-	-	-
Pseudomonas aerugionosa	1 (6.7)	-	-	+	-	-	-	-	-	-	-	-
Staphylococcus auerus	1 (6.7)	+	-	-	-	-	-	-	-	-	-	-
Total	15 (100.0)	4	2	4	-	3	-	-	-	-	-	2

Samples A, B, C and E are water samples from a borehole water supply. Sample D is a NAFDAC approved sachet water. Samples F, G, H, and J are well water samples. Samples I and K are river water samples.

sample D, F, G, H, I and J.

DISCUSSION

The pH values of the water samples are within the acceptable range; this conforms to the pH range reported by most authors (Okonko et al., 2008). According to Medera et al. (1982), the pH of most natural waters range from 6.5 - 8.5 while deviation from the neutral 7.0 is as a result of the CO_2/bicarbonate/carbonate equilibrium. The pH of brackish water bodies stated by Imevbore (1985) ranged from 6.5 - 7.4. The generally low pH values obtained in the lagoon water might be due to the high levels of free CO_2 in the water samples, which may consequently affect the bacterial counts. This was also reported by Edema et al. (2001) and Okonko et al. (2008) in similar studies. The pH of water is extremely important. The fluctuations in optimum pH ranges may lead to an increase or decrease in the toxicity of poisons in water bodies (Ali, 1991; Okonko et al., 2008).

This temperature range of 25 – 27.5°C reported in this study is comparable to the temperature ranges reported by other authors in a similar study. Alabaster and Lloyd (1980) reported a temperature ranges of 26 and 30°C in a similar study. This was attributed to the insulating effect of increased nutrient load resulting from industrial discharge. This temperature range of 25 – 27.5°C of water samples and the water body reported in this study is believed to have been influenced by the intensity of the sunlight as temperature rose from 28 - 30°C on relatively hot days (Mulusky, 1974; Banwo, 2006; Okonko et al., 2008).

The total counts for some of the water samples were generally high, exceeding the recommended standard limit for water (FAO, 1997; Okonko et al., 2008). Although the Lagoon is a flowing one, it is open to various objects uses and gross contamination as well as turbidity may result from the presence of high levels of organic waste matter. Recommended standard for water is nil (FAO, 1997; Okonko et al., 2008). The presence of coliforms group in some of these water samples generally suggests that a certain selection of water may have been contaminated with faeces either of human or animal origin. Other more dangerous microorganisms could be present (Richman, 1997; Okonko et al., 2008).

According to a study by Baxter-Potter and Gilliland (1988) on straight river water shed when precipitation and stream flows are high, the influence of continuous sources of pollution such as finding individual sewage treatment plants, industrial and institutional sources and waste water treatment facilities overshadows weather driven sources such as feed between run-off and urban storm water which leads to generation of faecal colliform concentrations. However, illegal dumping of domestic wastes, livestock management, faecal deposit and waste dumps also affect bacterial concentration in run-off (Okonko et al., 2008).

The isolated bacteria species were identified to be same with those commonly encountered in water and aquatic environments as was also reported in a study on streams surface water in Wyoming in U.S.A. reported by Clark and Norris (1999) and reviewed by Banwo (2006). These identified isolates include *B. cereus*, *E. aerogenes, Flavobacterium* sp., *Micrococcus* sp*., P. aerugionosa* and *S. aureus*.

The presence of enteric bacteria- *Micrococcus* sp. and *E. aerogenes* as reported in this study are indication of faecal contamination 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. Though the most frequently isolated index of water quality and indicators of faecal contamination such as *Escherchia coli* and *Streptococcus feacalis*, were not isolated in this present study, yet the presence of some indicator and other organisms examined is of special concern. The greatest danger associated with water used for food processing, drinking purposes and for human consumption is contamination by human excrement (Apantaku et al., 1998; Edema et al., 2001; Okonko et al., 2008). The need for microbial assessment of water for production of drinks should also be emphasized to re-

duce possible contamination (Fagade et al., 2005).

The presence of *Micrococcus* sp. and *E. aerogenes* reported in this study has also been reported by Umeh et al. (2005) in a study on the bacteriological quality and safety of pure water sold in Akwa, Nigeria using membrane filtration method. Umeh et al. (2005) reported the presence of enteric bacteria associated with fecal contamination and this include *S. faecalis*, *Citrobacter* sp., *Proteus mirabilis*, *Providencia* sp., *Micrococcus* sp., *E. coli*, *Shigella* sp., *E. aerogenes*, *Serratia* sp. and *Klebsiella* sp. Bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these microorganisms may pose a potential risk to consumers (Umeh et al., 2005).

Most of the organisms found on these water samples used for processing frozen seafood products in different processing plants are those commonly found in soil and water. The presence of *Micrococcus* sp. and *E. aerogenes* from these water samples is an indication of faecal contamination of the water used for processing frozen seafood products and might have adverse effect on the health of the consumers (Adebolu and Ifesan, 2001). Another organism found in these water samples is *S. auerus*, a pathogenic organism of public health concern and significance. The presence of this organism might have contaminated the water from source (Adebolu and Ifesan, 2001). 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.

This current findings of the unsafe water used for processing frozen seafood products are grim reminders of the need to address water and sanitation urgently in these environment. It is therefore necessary to recommend that public awareness programmes should be employed to educate owners of seafood processing plants, food processors, food vendors and general populace on the need for food safety and the requirement for water used for human consumption. Tap water, bornehole water, and publicly sold sachet water should be adequately treated before use. Well, tap, borehole and river water to be used for processing food purposes should be boiled and filtered where necessary before use in processing read-to-eat foods products for human consumption.

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