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Microbiological quality assessment and proximate analysis of fish and shrimps sold in open markets and grocery stores in Benin city, Nigeria

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SUMMARY: The aim of this research was to determine the microbiological quality and proximate composition of fish and shrimps sold in open markets and grocery stores in Benin City, Nigeria. Samples of fish and shrimps were analyzed microbiologically using pour plate isolation method. The total bacterial count/coliform count were7.80 ± 0.12 x 10^5/1.20 ± 0.13 x 10^5 and 5.44 ± 0.23 x 10^5/1.50 ± 0.11 x 10^5 for fish and shrimp samples respectively in the open market whilst for the grocery stores they were:3.61 ± 0.32 x 10^5/4.15 ± 0.33 x $10^5$and 1.42 ± 0.24 x 10^5/1.36 ± 0.13 x $10^5$for fish and shrimp samples respectively. The mean fungal count for fish and shrimp samples was highest in open market shrimps (2.11 ± 0.20 x 10^2) and lowest in grocery stores shrimp (1.33 ± 0.12 x 10^2). Bacterial species isolated were *Pseudomonas aeruginosa, Enterobacter aerogenes, Staphylococcus aureus, Micrococcus luteus* and *Staphylococcus epidermidis* while the fungi isolated were: *Aspergillus niger*, *Penicillium* sp and *Mucor* sp. Proximate analysis showed that the samples were mainly made up of protein, carbohydrate and lipids whilst moisture, fibre and ash were also present in all the samples at varying concentrations. Results revealed no significant difference in the proximate composition of the open market and grocery stores fish and shrimps. From the foregoing, these high sources of nutrients should be added to our daily meal, while proper measures, such as public enlightenment, washing and cooking of the samples with potable water to ensure their microbiological safety are recommended.

Keywords: coliform, fish, microbiological safety, proximate analysis, shrimps

Introduction

Seafoods is an important part of a healthy diet and one of the most important sources of animal protein source and other element for the maintenance of health body, however they are highly perishable food, with its quick perishability being

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the main problem during its preservation (Khan, 2001; Musa *et al*., 2010; Okoro *et al*., 2010; Dewi *et al.*, 2011; Renvichandran *et al.,* 2012). In handling and storage of fish, its quality deterioration rapidly occurs and truncates the shelf-life (Alemu, 2013). Fish contains proteins, minerals, vitamins as important sources of nutrients. However fish meat spoils more quickly than muscle foods, particularly via natural bacterial spoilage; about 30% of landed fish are lost through microbial degradation only (Ghaly *et al.,* 2010). Fish spoilage with microorganisms shows environmental pollution (Adeyemo, 2003). Therefore, the microbial biota of fish is a reflection of its aqueous environment. If the fish surrounding environment is polluted with bacteria, their consumption will be risky to human health (Arafat, 2013). Many researches of microbial flora in the body and internal organs of fish have been carried out (Al-Harbi and Uddin, 2004; Yagoub, 2009; Okoro *et al*., 2010; Das Trakroo and Agarwal, 2011; Adebayo-Tayo *et al.,* 2012a, b). These studies have revealed variation in the bacterial flora of fish species collected in different places and in different countries. Bacteria such as *Pseudomonas fluorescence*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Vibrio* sp. and Myxobacteria are common in the aquatic environment (Gilmour *et al*., 1976; Allen *et al.,* 1983). However, disease-causing bacteria are mainly introduced into water bodies through faeces from humans or animals (Arafat, 2013).Shrimp is one of the most delicious sea food and is part of the almost every nation's traditional meal (Ehigiator *et al.,* 2014)

Shrimps are found worldwide and they include commercially significant species such as the White leg shrimp, Atlantic white shrimp*,* Indian prawn and Tiger prawn. The shrimps constitute a large group of crustaceans varying in size from microscopic to about 35 cm long. The body is almost always laterally compressed, the rostrum usually compressed and toothed, and the abdomen long, longer than the carapace or head (Adedeji and Ibrahim, 2011).Shrimps can be pink, green, brown, blue, white or yellow before cooking but turn pink with white meat after cooking. Shrimps have suitable moisture contents (73.14% to 73.91%). The protein was found as the major constituent, indicating that shrimp muscle can be a good source of amino acids. Crude protein levels showed a tendency to increase in wild shrimp. The ash content in this study was a little higher than that obtained by Sriket *et al*. (2007).

Seafood refers to all fresh or salt water organisms such as shellfish, fin fish, mollusks, crustaceans and other forms of aquatic animal life. Nigeria has a large number of frozen seafood processing plants and retail marketsdistributed along the country, where considerate amount of people buy their frozen seafood product daily. The source of pathogenic bacteria may be from environmental cotamination or unhygienic handling of shrimp by the workers. When processed frozen sea foods are consumed raw, there is likelihood of endangering the health of consumers especially when the microorganisms present include pathogenic ones. (Okonko, 2008). The microbiological safety of food is achieved by ensuring the absence of

pathogenic microorganisms and by all means preventing microbial multiplication (Edema *et al*., 2005). Control and prevention of contamination in shrimps, through good water source, improved hygienic handling of shrimp , proper sewage disposal, proper storage of shrimp lead to reduction in shrimp (seafood) hazard and danger to public health (Ehigiator *et al*., 2014). Unsafe water used in processing seafood products pose a global public health threat, placing consumers at risk for a host of diarrhea and other diseases (Hughes and Koplan, 2005). Handling of raw materials influences the bacteriological quality of frozen shrimps. Insufficiently iced and improper storage of shrimp at higher temperature enhance the growth of microorganisms responsible for microbiological quality changes (Reilly *et al*., 1986).

Most of the methods that have been used to estimate the quality of fresh fish measure or evaluate parameters that are formed, varied or modified during deterioration of fish. These methods are either microbiological or chemical (Huss, 1995). Some of the microbiological methods used to assess fish freshness are total plate count, total coliform and fecal coliform. Total plate count is a good indicator of the sensory quality or expected shelf life of the product (Olafsdottir *et al*., 2006; Koutsoumanis and Nychas, 2000). A good knowledge of the microbial loads of raw processed seafood such as fish and shrimp is necessary so as to guide the unsuitability for consumption. Thus, regular microbiological analysis of seafood products at source or processing plant must be carried out to check for the effectiveness of the processes of processing and packaging. The study, therefore aims to determine the microbial load and the proximate analysis of fish and shrimps sold in the open markets and grocery stores in Benin City, Nigeria.

Materials and methods

Sample collection

Fresh fishes and shrimps were purchased in Oba market and grocery stores in Benin City. The samples were immediately transported to the laboratory for microbiological analysis.

Sample Preparation

Ten grammes of each sample *Clarias gariepinius* and *Caridina* sp.was of cut from the head, middle and tail regionswere weighed and mashed in a sterile laboratory mortar and pestle and aseptically introduced into 90 mL of sterile distilled water, properly shaken before a 10-fold serial dilution was prepared.

Preparation of culture media

All media were prepared accordingly to manufacturer's instruction. The media used in this study were Nutrient agar (used for heterotrophic bacterial count), MacConkey agar (used for coliform count) and potato dextrose agar (used for fungal count).

Isolation and enumeration of microorganisms

One millilitre from 10 dilutions was plated out by pour plate method on nutrient agar, MacConkey agar and potato dextrose agar. The nutrient agar and MacConkey agar plates were amended with nystatin to prevent fungal growth and then incubated at 37^0C for 24hrs. The potato dextrose agar plates were amended with streptomycin to prevent bacterial gowth and incubated at28^0C for 72hrs. After incubation, discrete colonies of culture on nutrient agar and potato dextrose agar plates were counted and expressed in cfu/g.

Characterization and identification of isolates

Bacterial isolates were identified on the basis of cultural morphological and biochemical tests according to Jolt *et al*., 1994 and Cheesbrough, 2006. The fungal colonies were identified as described by Harrigan, 1998.

Proximate Analysis

Proximate analysis of the samplefor moisture content, crude protein, lipid, fibre, ash and carbohydrate was determined using the methods description by AOAC (1990).

Statistical Analysis

Results were expressed as means ± standard error (SE) of three replicates. Data were subjected to Analysis of Variance (ANOVA) using SPSS version 16.0 (Ogbeibu, 2015).

Results and discussion

In this study, assessment of the microbiological quality and proximate analysis of fresh fish and shrimps sold in open markets and grocery stores in Benin City was carried out. Results showed that the microbial counts of both samples (Tables 1 and 2) were high.

Table 1.

Microbial counts of samples from open markets

Counts (cfu/g)	Fish	Shrimp
Total Heterotrophic Bacterial count	$7.80 \pm 0.12 \times 10^{5a}$	$5.44 \pm 0.23 \times 10^{5a}$
Coliform count	$1.20 \pm 0.13 \times 10^{5b}$	$1.50 \pm 0.11 \times 10^{5b}$
Fungal count	$1.56 \pm 0.21 \times 10^{2c}$	$2.11 \pm 0.20 \times 10^{2c}$

Note: Values are means ± standard error; Means with the same letter are not significantly different ($P > 0.05$)

Table 2.

Microbial counts of samples from grocery stores

Counts (cfu/g)	Fish	Shrimp
Total Heterotrophic Bacterial count	$3.61 \pm 0.32 \times 10^{5a}$	$4.15 \pm 0.33 \times 10^{5a}$
Coliform count	$1.42 \pm 0.24 \times 10^{5b}$	$1.36 \pm 0.13 \times 10^{5b}$
Fungal count	$1.61 \pm 0.41 \times 10^{2c}$	$1.33 \pm 0.12 \times 10^{2c}$

Note:Values are means± standard error; Means with the same letter are not significantly different ($P > 0.05$)

The total bacterial count for fish in open market and grocery stores were $7.80 \pm 0.12 \times 10^5$ and $3.61 \pm 0.32 \times 10^5$ cfu/g respectively. Fungal counts of $1.56 \pm 0.21 \times 10^2$ cfu/g and $1.61 \pm 0.41 \times 10^2$ cfu/g were respectively observed, while total coliform counts in both samples were $1.20 \pm 0.13 \times 10^5$ and $1.42 \pm 0.24 \times 10^5$ cfu/g respectively. The total bacterial load from open market and grocery store shrimps were $5.44 \pm 0.23 \times 10^5$ cfu/g and $4.15 \pm 0.33 \times 10^5$ cfu/g respectively. Fungal counts were: $2.11 \pm 0.20 \times 10^2$ cfu/g and $1.33 \pm 0.12 \times 10^2$ cfu/g respectively, while total coliform counts were $1.50 \pm 0.11 \times 10^5$cfu/g and $1.36 \pm 0.13 \times 10^5$ cfu/g respectively. This observation agreed with those of Ehigiator *et al*. (2014) and Alemu, 2013.The high microbial count could be attributed to poor handling and storage practices adopted by the sellers.

The counts were generally high and exceeded the FAO/WHO standard limit of 1.0×10^2cfu/ml for food production and water (FAO/WHO, 2007). In both open markets and grocery stores, the bacterial and coliform counts were higher than the fungal count. This difference could have been as a result of differences in preservation methods of the different markets and grocery stores and their storage conditions. Tables 3 and 4 showed the bacterial and fungal isolates respectively, as *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Micrococcus luteus*, *Staphylococcus epidermidis* (Table 3)*,* and *Aspergilus niger, Penicillium* sp., *Mucor* sp. (Table 4).

These results were similar to those reported by Ehigiator *et al.,* 2014 and Okonko *et al*. (2008). *Pseudomonas aeruginosa*was isolatedamong patients with wounds, burns and cystic fibrosis and their presence is likely due to the action of swimmers and infected individuals in water bodies and aquatic environments which they use for recreational purposes (Ehigiator *et al*., 2014). Presence of *S. aureus*, another pathogenic bacteria might be due to possible contamination during sales and unhygienic handling of seafood products. This is in agreement with the reports of Edema *et al.* (2005). Okonko *et al*. (2008 a and b) and Oluwafemi and Simisaye, (2005).

Table 3.

Bacteria isolated from fish and shrimps

Characteristics	*1*	*2*	*3*	*4*	*5*
Cultural					
Elevation	Low	Convex	Convex	Convex	Flat
Margin	Convex	Entire	Entire	Entire	Serrated
Colour	Green	Yellow	Yellow	White	Cream
Shape	Circular	Circular	Circular	Circular	Circular
Morphological					
Gram stain	-	+	+	+	+
Cell type	Rod	Cocci	Cocci	Cocci	Rod
Cell arrangement	Single	Cluster	Single	Cluster	Single
Spore stain	-	-	-	-	-
Biochemical					
Catalase	+	+	+	+	+
Oxidase	+	-	-	-	-
Coagulase	-	-	-	-	-
Urease	-	+	+	+	-
Indole	-	-	+	+	+
Citrate	+	+	+	+	+
Glucose	+	+	+	+	+
Lactose	-	+	+	+	+
Isolates	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Micrococcus luteus*	*Staphylococcus epidermidis*	*Enterobacter aerogenes*

Table 4.

Cultural and microscopic characteristics of fungal isolates

Cultural	**Microscopic examination**	**Isolates**
Black fluffy colonies with reverse side yellow	Septate and branched hyphae and conida in chains	*Aspergillus* niger
Grey colonies that were large with white border.	Long conidiophores consisting of broom like conida in chains	*Penicillium* sp.
White flat colony with reverse side colourless	Non-septate hyphae with straight sporangiophore with many spherical spores	*Mucor* sp.

The isolation of fungi in this study is similar to result obtained by Ehigiator *et al.,* 2014 and Fagade *et al*., 2005. Fungi might have arisen due to the fact that during storage, the samples reabsorb moisture from the environment, which supported the growth of these microorganisms. Table 5 displayed the distribution of isolates in the open market and grocery store. It was observed that *S. aureus* was the most prevalent in all the samples while the least prevalent was *E. aerogenes*. The presence of coliforms indicated faecal contamination of the water for processing the frozen seafood (Adebolu and Ifesan, 2001).

Table 5.

Distribution of isolates in samples

Isolates	Open Market		Grocery store	
	Fish	Shrimp	Fish	Shrimp
Pseudomonas aeruginosa	+	+	-	+
Enterobacter aerogenes	-	+	-	-
Staphylococcus aureus	+	+	+	+
Staphylococcus epidermidis	+	+	+	-
Micrococcus luteus	+	+	-	-
Aspergillus niger	-	+	+	+
Penicillium sp.	+	-	+	+
Mucor sp.	+	+	-	-

Key**:** + =Present, - = Absent

Proximate analysis (Table 6) of the different fish and shrimps samples showed the presence of protein, carbohydrates, lipid, moisture, fibre and ash in all the samples at varying concentrations. The protein content in fish samples from open market and grocery store was found to be slightly different and were 37.22 ± 1.23 % and 41.35 ± 0.76 % respectively in agreement with the results of Olayemi *et al*. (2011). The shrimp samples were found to be lower in protein content with percentage composition of 18.90± 0.79 % and 21.54 ± 0.81 % respectively in shrimps from open market and grocery stores. Interestingly, the carbohydrate content of shrimps was found to be very high compared to that of fish. A percentage composition of 50.77 ± 1.19 % and 46.17 ± 1.01 % were observed in the respective shrimps, compared to 16.41 ± 0.05 % and 13.71± 0.89 % in the respective fish samples. This is in agreement with the work of Puga-lópez *et al*. (2013) who reported similar findings on the proximate analysis of shrimps. The ash content of any sample is a measure of the mineral content of the food (Nnamani *et al.,* 2009). The ash content were: 7.43 ± 0.08 % and 2.32 ± 0.09 % for open market and grocery store fish respectively while 4.52 ± 0.49 % and 5.31 ± 0.44 % respectively for open market and grocery store shrimps. The moisture content for open market and grocery store fish were: 31.45 ± 1.41 % and 30.63 ± 0.87 % respectively, while 21.80 ± 0.92 % and 24.98 ± 0.89 % for open market and grocery store shrimp respectively.

Table 6.

Proximate composition of samples

Nutrients (%)	Open Market		Grocery store	
	Fish	Shrimp	Fish	Shrimp
Moisture	31.45± 1.41[a]	21.80±0.92[b]	30.63±0.87[a]	24.98±0.89[b]
Crude Protein	37.22± 1.23[b]	18.90±0.79[b]	41.35±0.76[b]	21.54±0.81[b]
Lipid	5.94± 0.11[c]	1.25 ± 0.11[d]	8.72 ± 0.19[c]	1.02 ± 0.08[d]
Fibre	1.55± 0.09[d]	2.76 ± 0.09[d]	3.27 ± 0.21[d]	0.98 ± 0.10[d]
Ash	7.43± 0.08[c]	4.52 ± 0.49[c]	2.32 ± 0.09[d]	5.31 ± 0.44[c]
Carbohydrate	16.41± 0.05[c]	50.77 ± 1.19[b]	13.71 ± 0.89[c]	46.17 ± 1.01[b]

Note:Values are means± standard error; Means with the same letter are not significantly different (P > 0.05)

Open market and grocery store lipid content for fish were: 5.94 ± 0.11 % and 8.72 ± 0.19 % respectively, while 1.25 ± 0.11 % and 1.02 ± 0.08 % for open market and grocery store shrimps respectively. The fibre content for the open market and grocery store fish were: 1.55 ± 0.09 % and 3.27 ± 0.21 % respectively, while 2.76 ± 0.09 % and 0.98 ± 0.10 % for open market and grocery store shrimps respectively. Results revealed no significant difference in the proximate composition of the open market and grocery stores fish and shrimps. This finding suggests that fish and shrimps are very high in nutrient composition and should be included in our meals.

Conclusions

The microbiological and proximate analysis of fish and shrimps sold in open markets and grocery stores have been evaluated in this research. Results revealed that the samples exceeded the acceptable standard limit of contamination recommended by FAO/WHO and also microorganisms identified in this study could pose high health risk. It is recommended, therefore, that both open market and grocery store fish and shrimps be properly washed and cooked adequately before consumption. Public enlightenment and proper monitoring by food regulatory bodies are also recommended.

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