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MICRO-ORGANISMS ASSOCIATED WITH SMOKED PRAWN (*Macrobranchium* spp.) IN SELECTED MARKET LOCATIONS IN ABEOKUTA METROPOLIS OF OGUN STATE, NIGERIA

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ABSTRACT

This study is aimed at isolating and characterizing micro-organisms of *Macrobranchium* spp. gotten from markets in Abeokuta. Twelve different samples of whole smoked prawns (*Macrobranchium* spp.) were purchased from two different locations each in six major markets (Itoku, Omida, Iberekodo, Lafenwa, Panseke and Olomore) within the Abeokuta metropolis in Ogun State. Their microbial load was analyzed using Mac-Conkey agar (MA), Deoxycholate citrate agar (DCA), Nutrient agar (NA), and Mannitol salt agar (MSA) for bacteria isolation while Potato Dextrose agar (PDA) was used to isolate the fungi in the microbiology laboratory of the department of Microbiology, Federal University of Agriculture, Abeokuta. *Staphylococcus aureus* and *Citrobacter* spp (22.22% each) dominated the samples while the fungal specie that occurred most frequently in the samples was *Aspergillus niger* (31.03%). The total bacterial counts for all the samples ranged from $9x10^2$ to $1.0x10^3$ cfu/g and fungal count were between 21%-90% in terms of frequency of occurrence. These microorganisms cause food spoilage and poisoning and the occurrence of such microorganisms may be as a result unhygienic handling of prawns during processing as some of the microorganisms may be post-harvest contaminants. Adequate cooking could help in reducing microorganism of smoked prawn.

Keywords: Bacteria, fungi, microbial load, smoked prawn, spoilage.

INTRODUCTION

Food, fish and other aquatic product's insecurity in developing and under developed countries has led to the evasion of some diseases attributable to the consumption of these products. Seafood however refers to all fresh or salt water organisms such as shellfishes, fin fishes, mollusks, crustaceans and all other forms of aquatic animal life.

Prawn, a source of animal protein is one of the commonest in Africa and Asia countries. The short supply of animal protein in Nigeria to a level almost beyond the reach of the low income earners has also made this group of seafood an alternative source of animal protein. Prawns are low in fat and calories, contain a lot of omega-3 fatty acids, a high level of vitamin B_{12}, zinc, iodine, phosphorus, potassium, selenium and iron but have smaller quantity of magnesium, calcium and sodium (Food and Drug Admini-

stration, FDA, 2007). Shrimp continues to represent one of the safest forms of muscle protein consumed in the world. Amongst seafood, it is possibly the least problematic product in terms of reported illnesses per volume consumed (Otwell and George, 2010).

In order to prevent spoilage and increase shelf life of fresh prawn, value adding through further processing (e.g. smoking) is necessary. Smoking is one of the traditional fish processing methods aimed at preventing or reducing post-harvest losses. Unfortunately, much of the prawn smoked today is exposed to smoke just long enough to provide the desired flavor with little if any drying (Chinivasagama *et al.*, 1996; Piggot, 2009), making the prawns suceptible to spoilage. Processing of a fish species and shell fish inevitably entails a storage period for the finished product prior to marketing and consumption. Since fish are composed of perishable nutrients, storage period should be kept to a minimum with adequate storage conditions provided so as to prevent deteriorative changes occurring through oxidative damage and/or microbial, insect or rodent infestation. The most important environmental factors governing the storage or shelf life of fish are ambient temperature and humidity. These factors dictate the rate at which chemical changes take place (Daramola *et al.,* 2007). In the present research an attempt has been made to investigate the micro-organisms associated with smoked prawn (*Macrobranchium spp.*) in selected market locations in Abeokuta metropolis of Ogun State.

MATERIALS AND METHODS

The Study Area

The study was carried out in Abeokuta metropolis, comprising of Abeokuta North and Abeokuta South Local Government Areas of Ogun State in Nigeria. Good majorities of the people in these local government areas are farmers, traders and civil servants as well as non-farm workers. Abeokuta metropolis has a hot humid climate with annual rainfall of about 1200mm. It is in the tropical rain forest zone of Nigeria. The rural farming population estimated for Abeokuta metropolis as at 1993 was 34,262 and this gives a projected figure of 55,431 by the year 2010, given the population growth rate of 2.83 percent (OGADEP, 1993; FGN, 1997; FMA, 1997). The study area is part of Abeokuta zone as classified by Ogun State Agricultural Development Programme (OGADEP). The state has a total water surface area of 2,237,000 hectares (Ita *et al*., 1984) and land area of 16,369,370 square kilometers. The Abeokuta zone of unified extension services was purposively selected due to the fact that fish farming business are majorly embarked upon by the people in the zone (Olaoye *et al.*, 2007).

Sampling Procedures and Sample size

Ready-to-eat smoked dried prawns were purchased from 6 different markets; Lafenwa, Olomore, Kuto, Itoku, Iberekodo and Panseke. A total number of 12 samples were collected [2 from each location] and kept in cellophane bags and transported to the laboratory. The samples collected included the carapace, entails and the exoskeleton of the prawn (*Macrobranchium* spp.).

Preparation of sample for culture

Ten (10) grammes of the whole prawn sample for microbiological evaluation were weighed into 9ml of sterile distilled tap water in the bijou bottle. This was done for samples gotten from each location and was taken as the original stocks sample of each market

location. 1ml of the original stocks solution was poured into 9ml sterile distilled water and mixed thoroughly to give 10^{-2} of the original sample making a tenfold serial dilution.

Isolation and characterization of bacterial isolates

Samples were inoculated on Mac-Conkey agar (MA), Deoxycholate citrate agar (DCA), Nutrient agar (NA) for isolation of organisms, Mannitol salt agar (MSA) for selective isolation of *Staphylococcus* and Potato Dextrose agar (PDA) for isolation of fungi. Inoculated plates were incubated aerobically for 24hours at 37^0C while fungal culture plates containing acidified PDA were incubated at 25^0C for 3-7days.

Colony counts were done using digital unlimited colony counter and counts expressed in coliform forming unit per gram (cfu/g) of the sample. The bacteria colonies were sub cultured on fresh media and identification was done using standard procedure and biochemical tests such as gram staining, catalase test, citrate utilization test, sugar fermentation test, coagulase test, indole test and oxidase test (Fawole and Oso, 2001, Jimoh *et al*., 2009, Olutiola *et al*., 1991, Taylor and Stone, 2008).

Isolation of fungi

The samples were inoculated on PDA and incubated at room temperature for 3days. After incubation, plates were sub cultured on freshly prepared PDA agar to get pure culture of the organisms (Ibrahim and Rahma, 2009).

Identification of fungi

This was done according to James and Natalie (2001) using cotton blue in lactophenol stain. The identification was done by placing a drop of the stain on a clean slide with the aid of a mounting needle; a small portion of the mycelium from the cultures was removed and placed in a drop of lactophenol. The mycelium was spread on the slide using a needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide the mounted and viewed under x10 and x40 objective lenses respectively. The species observed were identified according to Cheesbrough (2000), Ibrahim and Rahma, (2009).

Data Analysis

The data from this study were analysed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) and further presented using tables, percentages and figures.

RESULTS AND DISCUSSION

The total count (in CFU/g) of bacteria and fungi present in the ready-to-eat prawns is given in Table 1. The total counts were generally high and it ranged from $1.1x10^3$ to $9x10^2$cfu/g. Samples from locations 1 and 3 (Kuto and Lafenwa) possessed the highest bacteria load (22.04% and 22.95%) respectively; locations 2 and 5 (Iberekodo and Olomore) had the least bacteria load of 10.63% & 9.56% respectively.

Though, location 3 had a higher bacterial load as compared with location 1 but there was no significant difference between the bacterial loads of the samples from the two locations. Similarly, location 3 (Lafenwa) had the highest load of fungi (32.89%), followed by samples from location 2 (Iberekodo 22.09%), samples from 4 had the least fungi load.

Table 1:- Microbial load on prawn samples (cfu/g)

Sample code	NA		MSA		MA		PDA		Suspected organism
S1A	2.14X 104	2.13X 104	3.40 X 103	3.40X 103	2.14X 104	2.16X 104	2.40X 103	2.50X 103	S.aureus, P. aeruginosa, Shigella spp, Citrobacter spp, A.niger, Serattia spp, Klebsiella spp,Penicillium spp, C. krusei, A. oryzae
S1B	2.15X 104	2.14X 104	3.3X 103	3.40X 103	2.16 X 104	2.14X 104	2.30X 103	2.40X 103	S.aureus, P. aeriginosa, Shigella spp, Citrobacter spp, A.niger, Serattia spp, Klebsiella spp, Penicillium spp, A. Oryzae
S2A	8.00X 102	7.00X 102	9.00X 102	8.00X 102	1.20X 103	1.20X 103	9.00X 102	9.00X 102	S.aureus, B.cereus, Proteus vulgaris, E.coli, Citrobacter spp, A.niger, Mucor spp
S2B	8.00X 102	8.00X 102	8.00X 102	8.00X 102	1.1 X 103	1.2 X 103	9 X 102	8.00X 102	S.aureus, B.cereus, Proteus vulgaris, E.coli, Citrobacter spp, A.niger, Mucor spp
S3A	2.42X104	2.39X104	3.00X102	3.10X103	2.64X104	2.66X104	1.63X104	1.66X104	S.aureus, P.aeriginosa, Penicillium spp, E.coli, Citrobacter spp, A.niger, Mucor spp ,klebsiella spp, Serattia spp, C.krusei, Rhizopus spp, A. Flavus
S3B	1.21X104	2.43X104	2.9X103	3.00X 103	2.68X 104	2.67X 104	2.40X 103	1.68X 104	S.aureus, P.aeriginosa, E.coli, Citrobacter spp, A.niger, Mucor spp ,Klebsiella spp, Serattia spp, C.krusei, Rhizopus spp, A. flavus, Penicillium spp
S4A	1.21X104	1.24X104	2.00X102	2.00X102	2.82X104	2.86X104	2.10X103	2.10X103	S.aureus, Citrobacter spp, A.niger, Serattia spp,Pseudomonas spp, Penicillium spp
S4B	1.20X104	1.22X104	2.00X102	1.00X102	2.96X104	2.88X104	2.10X103	2.10X103	S.aureus, Citrobacter spp, A.niger, Serattia spp, Pseudomonas spp, Penicillium spp, Mucor spp,
S5A	7.00X102	7.00X102	8.00X102	8.00X102	9.00X102	1.2X103	1.2X103	9.0X102	S.aureus, Citrobacter spp, A.niger, Serattia spp, A.flavus, Proteus vulgaris
S5B	8.00X102	7.00X102	7.00X102	8.00X102	9.00X102	1.20X102	1.30X102	8.00X102	S.aureus, Citrobacter spp, A.niger, Serattia spp, A.oryzae, Mucor spp,Pseudomonas spp
S6A	2.14x103	3.00x103	2.18x104	2.40x102	3.10x103	3.33x104	2.60x103	2.66x103	S.aureus, Citrobacter spp, A.niger, Serattia spp, A.oryzae, Penicillium spp,C.krusei
S6B	2.42x104	2.14x103	1.20x104	2.43x104	2.90x104	3.10x103	2.60x103	2.10x103	S.aureus, Citrobacter spp, A.niger, A.oryzae, Penicillium spp, C.krusei, Mucor spp, B.cereus

Key: NA: Nutrient agar, PDA: Potato dextrose agar, MSA: mannitol salt agar, MA:Mac - Conkey agar, NIL: No count, CFU/g:colony forming unit/gram, E:*Escherichia*, S:*Staphylococcus*, A:*Aspergillus, spp.*: specie, P:*Penicillum*, R:*Rhizopus*, A: first market location, B: second market location

Though, location 3 had a higher bacterial load as compared with location 1 but there was no significant difference between the bacterial loads of the samples from the two locations. Similarly, location 3 (Lafenwa) had the highest load of fungi (32.89%), followed by samples from location 2 (Iberekodo 22.09%), samples from 4 had the least fungi load.

Bacteria from nine different genera were isolated and identified from the prawn samples obtained from the 6 market locations in Abeokuta (Tables 1 & 2). *Staphylococcus aureus* and *Citrobacter spp*. (Figure 1) dominated the samples (22.22% each) this is a confirmation of the work of (Okonta and Ekelemu, 2005) who reported *Staphylococcus* as one of the predominant micro-organisms affecting smoked fish and causing their spoilage, *Bacillus cereus* (3.70%) occurred least in the samples. *Bacillus* is the normal microbial flora of the fish and are not initially harmful, as they help in preventing the invasion of the fish flesh by other micro -organisms but may become pathogenic as a result of poor handling, poor hygiene and delayed processing and preservation of the prawn after harvest, other bacteria species occurred sparingly. The presence of *Staphylococci* is usually indicative of contamination from the skin, mouth or nose of food handlers (Jimoh *et al*., 2009). Inadequately cleaned equipment or raw animal products may also be a source of contamination. The presence of large numbers of bacteria in the collected samples in the study areas was a good indication of poor hygiene and temperature control.

The presence of substantial numbers of *E. coli* in foods suggest a general lack of cleanliness in handling and improper storage. *Bacilli* spp. was only isolated from prawn samples from locations 2 and 6. It is a gram-positive, obligate aerobe rod shaped, endospore forming bacteria (Todar, 2008). Two *Bacillus* spp. are considered medically significant*; B. anthracis* which causes anthrax and *B. coagulase* also causes food spoilage. Colonially, they are large, spreading and irregularly shaped. When viewed under microscope, they appear as rods with a bulge which contains the endospore (Martinko, 2005).

Among the fungal isolates, *Aspergillus niger* had the highest percentage of occurrence of 31.03%, while *Rhizopus* specie had the least percentage of occurrence of 6.90% (Figure 2). Other fungi family: *Penicillium* (20.69%), *Aspergillus oryzae* (13.79%), *Aspergillus flavus* (6.90%), *Mucor spp.* (20.69%) occurred sparingly in the samples. Only one class of yeast (*Candida krusei*) was isolated and identified from the samples. The presence of micro-organisms in these samples might be the result of contamination during sales or unhygienic handling of the prawns by the sellers who display the products in the open market places without covering them. In a similar study conducted by (Edema and Agbon, 2010), on the significance of fungi associated with smoked-cured *Ethmalosa fimbriata* and *Clarias gariepinus*, it was observed that the nutrient value of these smoked cured fish is not significantly diminished by the smoking process but that the economic value may be determined by the quality of the fish presented for sale.

Location 3 (Lafenwa) had the highest load of fungi (32.89%), followed by samples from location 2 (Iberekodo: 22.09%), then samples from 4 had the least fungi load. It was also observed in this study that the presence of fungi particularly aflatoxigenic moulds in these fish species is very significant from a food safety point.

Table 2: Morphological, biochemical and tentative identification of bacteria from prawn samples

Gram reaction	Citrate utilization test	Catalase test	Coagulase test	Motility test	Indole test	Glucose	Lactose	Sucrose	Suspected organisms
+	+	+	-	-	-	AG	AG	AG	Staphylococcus aureus
-	+	+	+	+	-	AG	-	-	Pseudomonas aeruginosa
	-	+	-		-	A	A	A	Shigella spp
-	+	+	-	+	-	AG	-	A	Citrobacter spp
-	+	-	-	-	-	AG	+	+	Klebsiella spp
+	+	+	-	-	-	-	+	+	Bacillus cereus
-	-	+	-	+	+	AG	AG	AG	Escherichia coli
-	+	+	-	+	-	AG	-	AG	Proteus vulgaris
-	-	-	-	+	-	AG	-	AG	Serratia spp

Figure 1: Percentages of occurrence of bacteria spp. present in the prawn samples

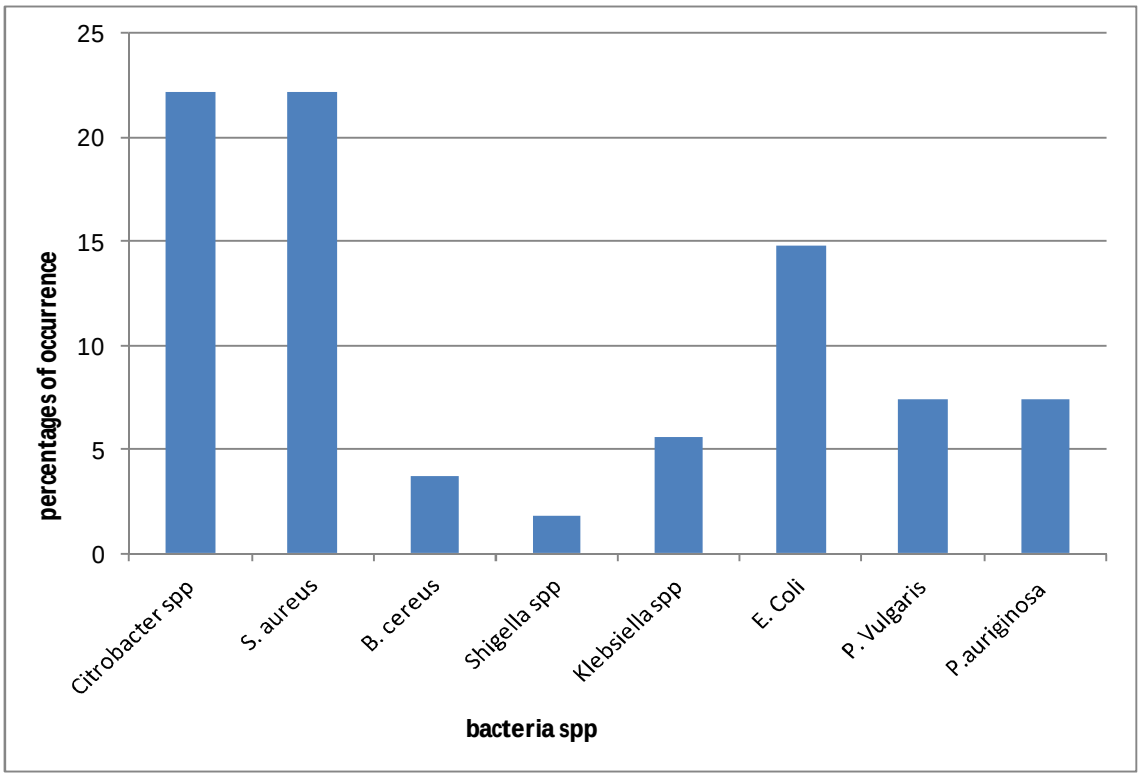
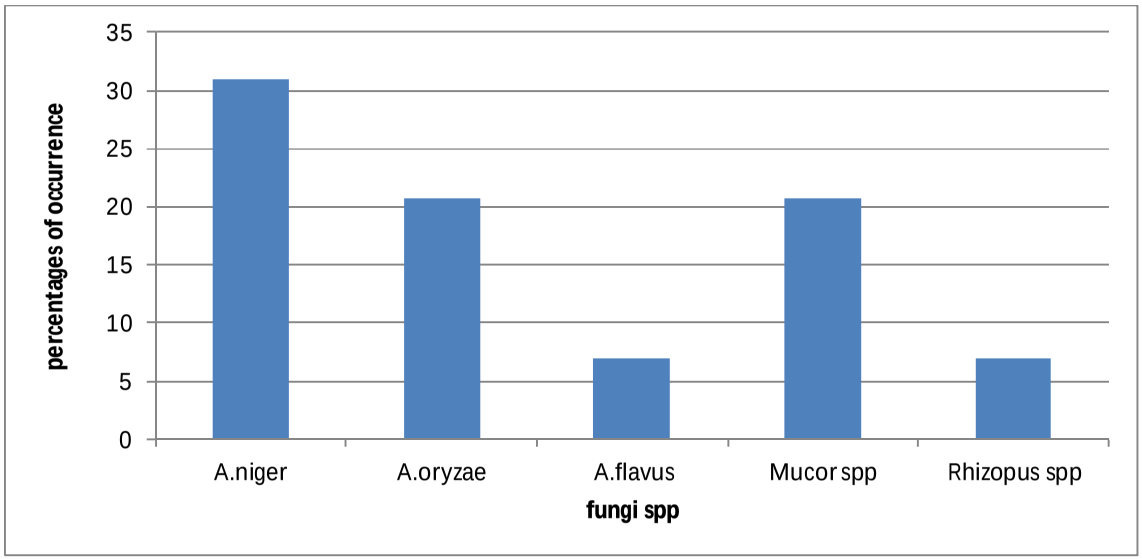


Table 3: Cultural and morphological characteristics of fungi isolated from the prawn samples

Shape	Surface	Elevation	Spore colour	Type of mycelium	Type of reproduction	septation	Appearance of special structures	*Suspected microorganisms*
Filamentous	Powdery	Raised	Black	Conidiospore	Sexual	septate	Foot cells	*Aspergillus niger*
Circular	Fluff	Raised	Greenish Blue	Conidiospore	Sexual	Septate	-	*Penicillum spp*
Circular	Powdery	Semi-Raised	Black	Conidiospore	Sexual	Septate	-	*Aspergillus oryzae*
Filamentous Woven Mass	Cotton	Raised	Black	Sporogiospore	Asexual	Non-septate	Rhizoid	*Rhizopus spp*

Figure 2: Percentage of occurrence of fungi species present in the prawn samples



CONCLUSION

In conclusion, Prawns which serves as an alternative source of animal protein cannot be banned from consumption based on its nutritional values; hence efforts should be made by relevant organizations on the formulation and enforcement of laws which would promote proper post-harvest technologies and good hygiene practice of prawns and other seafood products. Also, efforts should be geared towards awareness programs amongst food vendors about safe and hygienic practices and its importance to the health of man. Consumers also need to recognize that a healthy food means a healthy heart hence, prawns and other foods purchased from open markets should be processed further through washing and heating.

RECOMMENDATIONS

It is hereby recommended that

i. Smoked prawn packaging should be fly-proof in order to prevent the invasion of microbial growth on the products;

ii. Proper handling, processing and preservation of the prawns and other sea foods must be ensured; and

Consumers of smoked prawns and other sea foods should clean wash and subject these products to further cooking or heating so as to destroy all heat labile microorganisms present.

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