MICRO–ORGANISMS ASSOCIATED WITH SMOKED CURED FRESH WATER PRAWN IN OPEN MARKETS IN ABEOKUTA, NIGERIA.

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ABSTRACT

This study aimed to isolate and characterize microorganisms of smoked cured *Microbranchium.* Examination of smoke prawn sold in the four (4) different markets in Abeokuta (Itoku, Lafenwa, Kuto and Omida) revealed the presence of micro–organisms, with the intestine having the highest range of bacteria load, followed by the skin, while the carapase had the least. The bacteria load ranged from $2.0x10^4$g in the gill of smoke hake prawn from Lafenwa market to $9.3x10^5$g in the intestine of smoke panaeidae from Omida market. The following species of micro-organisms were detected: *Escherichia coli* and *Proteus spp.* with *Micrococcus spp.* and *Bacillus spp.* being the least occurring. While some samples from Elega markets harboured five different types of bacteria. *Rhodo-oorula spp.* and *Candida spp.* were more prevalent on smoke prawn than molds (*Aspergillus spp.* and *Penicillium spp.*). These micro–organisms cause food spoilage and poisoning. Some are probably post-processing contaminants. Local method of processing (smoking) fish seems to be inadequate for prolonging their shelf-life, thus; the need for better and improved processing techniques.

Keywords: Smoked, Prawn, Microbial load, Open Markets and Abeokuta

INTRODUCTION

Macrobrachium species continue to receive tremendous attentions as it supports very important local fisheries in Nigeria and other regions of Africa (Jayachandran, 2001; Akintola *et al.,* 2009). Ice against traditional smoking for fish products is becoming more popular. However, irregular supply of electricity in the country hampers the use of ice as a means of preserving fish products in spite of the high profile of ice globally as the cheapest and commonest method of prawn preservation (Kirschnik, *et al*., 2006).

Prawn As Protein Source

Prawn offers quality protein, low saturated fat, may contain Omega-3 (Ω-3) fatty acids and contribute to cardiovascular stability of adults as well as children's growth and development (FDA, 2007). Rapid spoilage due to higher water content and other issues mentioned in Abu-bakar *et al*., (2008) are important concern and affect shelf-life of the prawn.

Shelf-life

The shelf-life depends on the numbers and types of microorganisms, mainly bacteria, initially present and their subsequent growth as well as natural sources (Abu-bakar *et al.,* 2008). Shelf-life reported for whole *M. rosenbergii* stored on ice, included 3 days

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(Nip and Moy, 1988), 8 days (Lindner *et al*., 1988), 7-10 days (Kirschnik, *et al*., 2006), 14 days (Abu-bakar *et al*., 2008).

Lalitha and Surendran (2006) reported reduction in bacteria load after 5 days due to the inability of some of the bacterial species to survive and/or grow at low temperatures. Studies on the impact of ice on the qualities and safety of other freshwater prawn of genus *Macrobrachium* are rare in spite of their widely acknowledged status as species of ecological and economic importance to many fisheries.

Composition of Prawn

Prawn (particularly shell spine-prawn) on the average is composed of 9 – 35 mg of glucose, 10 – 60 mg of glycogen, 15 – 20% protein, 1.5% extractives, 1/5% ash, 0.2% fat and 60 -80% water (Shewan and Jones, 1957) prawn protein, like other animal protein contain most of the essential amino acids especially those that are lacking in protein of plant origin namely lysine. Methionine and tryptophan, and for this reason prawn protein is described as first class protein, the consumption of which is highly desirable.

Other Use of Prawn Product

- Prawn oils, besides being used as food are used in the soap manufacturing industries.
- The shell of some prawn yields substances that when coated on the inside of glass bead make artificial pearls.
- Some whole prawn and other species not really acceptable for direct consumption are converted into prawn meal for the manufacture of animal feeds.

Economic Loss of Prawn

A major economic loss is physical deterioration due to spoilage from improper handling or mechanical breakdown. This may result in the total or partial loss of the catch and temporary economic hardship for the fisherman or distributor. Economic losses can also occur from an over abundance of catch especially of seasonal prawn. Lack of cold storage and processing capabilities may cause the market to be flooded drastically reducing the market value of the prawn and negating profit to the people that catches it. Further economic losses occur in the sea food meant for exportation, such as highly priced shrimps. These products are rejected due to bacterial contamination, presence of heavy metals, chemical or pollutants or because they fail to meet the quality standard of the importing country (Pillai, 1957).

Nutritive Loss of Prawn

Nutritive losses with regards to post harvest prawn loses refer to prawn that have decomposed to such a degree that they are unfit or unsafe for human consumption. The improvement in the quality of fresh prawn and prawn products in less developed countries will help to reduce post harvest losses thereby present a more palatable product to the consumer and create new markets and employment opportunities (Poulter *et al*., 1987).

History of Prawn Smoking

Prawn preservation had existed longer than any food preservation technique. Fresh water prawn shell found in cave dwelling (inhabited 20,000 years ago) which is many days walking distance from the coast; of the same form of curing, smoking, salting and drying have continued unaltered from pre-history to the present day. Modern developments have centered on understanding and controlling the process to achieve the standardized product demand by today's market (Poulter *et al.*, 1982).

Economic Importance of Prawn Smoking

Consumers acquired a taste for smoked prawn as a pleasurable alternative to the consumption of fresh prawn. Another, function of prawn smoking is wide spread use as means of prolonging the shelf- life of unsold prawn after retail display. Preservation of prawn is still the prime objective of prawn smoking in most parts of the world. Having a shelf–life of several years, produced by repeated salting, boiling, drying, smoking, pressing and re-boiling of prawn (Poulter *et al.*, 1982).

POST HARVEST PRAWN TECHNOLOGY

Fresh Prawn

In as much as a major portion of all prawn in the fresh state serves as raw materials for all processed sea foods, reduction of post harvest losses at this stage is of fundamental importance. Typical of this is the recommended international **"Code of Practice for Fresh Prawn"** published jointly in 1976 by the Food and Agriculture Organization and the World Health Organization. In their recommendations a great deal of emphasis is placed on the need for cleanliness and coding in order to prevent bacteria spoilage and maintain freshness.

Prawn Preservation and Processing

Methods and technological advancement in prawn preservation must recognize some of the limitations of prawn as food. Prawn is much, more to contend with, noting the fact that not all prawn has the same processing and storage characteristics. This, to a greater extent than in the processing of beef and poultry, the biochemical properties of individual prawn species must be taken into account when deciding how they will be handled, processed and marketed (Horner 1991).

Refrigeration of Prawn

Refrigeration plays a major role in prawn preservation worldwide with the continued expansion of refrigeration facilities in both developed and developing countries some evolutionary changes in refrigeration systems themselves, now taking place, will help to ensure wider application. Refrigeration systems are becoming more reliable and easier to use. In recent years, the screw compressor has been introduced into the large installation in favour of the reciprocating compressor.

Micro–processors are being used to control and monitor industrial refrigeration systems. Frith and Heap (1987) have given details of a prototype microprocessor for transport refrigeration unit. In a review of these and other current trends, Stoecker (1987) has described the safety and environmental issues associated with refrigerants – chlorofluorocarbons and ammonia.

Cured Prawn

Smoked and salted prawn has traditionally been widely accepted and has broad international market appeal. The technology associated with these processes has changed over the years. Some improvements have been made in the operation of mechanical smoking kilns, especially better controls and automatic smoke producers. Also it has been feasible to use liquid smoke in some applications. One fact that is often ignored is that, for extended storage life, smoked prawn products should be frozen to protect quality except for hard-cured items. It has received insufficient attention and there is need to improve the drying technology through the utilization of heat pumps, waste heat and solar energy. A project supported by Canadian International Development Research Center was carried out at the University of Rhode Island wherein a plot model heat pump prawn dryer has been designed and equipped with a computer for control and data collection (Carpio and Merritt, 1987). Through the software, the dryer can be operated in different modes for control of air temperature and humidity constant variable with time and variable according to drying rate.

Prawn Spoilage

Over 10 to 25 years, as a generalization, the global emphasis has been on the increased production of prawn through traditional capturing of prawns. More recently it has also been recognized that aquaculture will play an increasing role in providing prawn for human consumption FAO, however, estimated that by the year 2000 the demand for prawn will be in excess of about100 million tons.

Prawn has been described as one of the most perishable of all stable commodities and post harvest losses are generally accepted to be high (U. S. National Academy of Science, 1978).

Spoilage of Processed Prawn

This section will concentrate on cured prawn that is, salted, dried and smoked. Very few investigations have been carried out on the spoilage of other traditional prawn products such as fermented, boiled, marinated prawn etc.

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Further economic losses occur in the sea food meant for exportation, such as highly priced shrimps. These products are rejected due to bacterial contamination, presence of heavy metals, chemical or pollutants or because they fail to meet the quality standard of the importing country (Indirati *et al.,* 1985).

Nutritive Loss of Prawn

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Challenges of Processing Prawn

Curing of prawn correctly undertaken with appropriate drying conditions is an excellent means of preserving prawn. Problems and losses, however, can occur. These are largely due to adverse weather conditions preventing the rapid reduction of water activity required to prevent spoilage. Such problems are more severe when salt is not used in the processing method. Thus in Malawi, losses are low during the dry season when the relatively low humidity and long periods of sun- shine mean that unsalted prawn spread on racks dries quickly. However, as the rainy season approaches the ambient humidity rises and drying is slow.

Under certain circumstances, improved processing method may not be effective in reducing physical losses and it then became necessary to consider the use of insecticides to control insect infestation in cured fish (Horner, 1991). The hemicelluloses are the least heat – stable of the wood components and readily decompose to yield furan and its derivatives together with a range of aliphatic carboxylic acids. The hemicellulose contents of hardwood have higher pentosans than in softwoods while hexosans may sometimes be greater in softwood. This might give hardwood smoke with greater preservative power and a more stable product hence the traditional preference for hardwood for smoking.

Smoke Composition

Smoke is composed of two phases – a particulate or dispersed phase and gaseous or dispersing phase. The dispersed particles are of the order of 0.196 to 0.346 micron (μ) average diameter. Dispersed particles liquid droplets formed by condensation of smoke components and make up the visible parts of smoke. These smoke particles contain different substances such as tars, wood resins and other compounds of high and low boiling points, which vary depending on the temperature at which smoke is generated and the smoke concentration. The gaseous or dispersing phase of smoke contains most of the flavour components such as the phenols.

During thermal degradation or pyrolysis of wood quite a large number of chemical compounds are produced. At the high temperature at which pyrolysis takes place, these compounds are gaseous. As these gases cool and mix with air, the less volatile compounds condense on nuclei forming the smoke particles and some of the more volatile compounds dissolve in these nuclei. The smoke constituents are now partitioned into phases, the dispersed liquid–phase smoke particles and a dispersing vapour phase. When smoke vapours are absolved by foods equilibrium is maintained by the release of absorbed compounds from the dispersed phase. These absorbed components in the smoke vapours gives raise to the characteristic colour, flavour and preserving properties of smoke foods (Howgate, 1984).

Over 300 chemicals compound have been detected in wood smoke and liquid smoke preparations. Modern methods of chemical analysis such as infrared (IR), mass spectroscopy (MS), gas chromatography (G.C.), and nuclear magnetic resonance (NMR) have been used in the identification of these compounds. There are more than 45 phenols, 70 carbonyls, 20 acids, 11 furans, 13 alcohols and esters, 13 lactones and 27 polynuclear aromatic hydrocarbons in smoke (Shewan and Jones, 1957).

Storage and Transport Challenges

A number of factors affect spoilage during storage and transport. Sometimes spoilage may largely be avoided by ensuring a rapid sale without allowing sufficient time for prawn to spoil. For instance, near some big cities, where there is a good market for prawn, it is possible to process prawn by a short period of hot smoking. This gives the product a shelf life of a few hours /days such that the product can be sold locally without serious loss. On the other hand storage following processing is often necessary when prawn is landed in areas remotes from the market as to even out fluctuations in supply. This can be a major cause of loss (Poulter *et al.*, 1987). Even in countries with low ambient relative humidities, losses during storage can be high due to insect pest infestation. As described above. This is particularly a problem in unsalted prawn. To keep losses to a minimum the cured prawn should be distributed and sold as soon as possible. In remote areas, however, this is not always possible since transport may only be available periodically and it is often necessary to accumulate sufficient materials to fill a complete pack. In addition, roads are often in bad condition and possibly impassable for part of the year due to floods etc. Under these conditions, storage losses can be very high (Green, 1967).

Wood smoke contains various phenolic compounds which are believed to have an insecticidal effect. Repeated smoking will increase the content of the phenols but it should be noted that it will also increase the concentration of polynuclear aromatic hydrocarbons which are believed to be carcinogenic (Obileye and Spinell, 1978). The hot condition in which smoking is conducted in many countries will destroy any egg or larva already in the prawn and destroy any adults from further quipositition. Hot smoking reduces the moisture content of the prawn and thereby renders it too dry to attract further infestation by blowflies. However, smoked prawn do become infested with beetles during subsequent storage hence any protection offered by the phenolic compounds appear to be minimal. The aim of this study is to isolate and characterize microorganisms associated with smoked cured *Microbranchium spp.*

MATERIALS AND METHODS

Sample Collection

The following markets were randomly selected; Kuto, Omida, Lafenwa and Itoku all in Abeokuta, Ogun State, Nigeria. A total number of 108 samples were collected, 27 samples were collected from each market. The samples were collected from the carapace, entails and the exoskeleton of Prawn (*Macrobranchium spp.*).

Samples Preparations and Analysis

2g of each sample was extracted. Samples were inoculated on Blood agar, MacConkey agar and Deoxy Cholate agar plates, for the primary isolation of organisms. Selenite–F broth; a liquid selective medium were also employed for the selective growth and isolation of salmonella species. All the plates were incubated at 37^0C overnight and subculture made from selenite–F broth was incubated in the same way. Suspected isolates from culture were identified.

Identification of Microorganism

The organisms were identified using Grams reaction and biochemical tests such as Catalase, Coagulase, Citrate Utilization, Urease, Nitrate Reduction, Indole Reaction, Oxidase, Motility, Sugar Fermentation tests and so on were carried out according to Akinyemi (2009) and Olutiola *et al.,* (1991) to identify sample by their reaction to the tests.

Data Analysis

The data generated from this study was analyzed using Microsoft Excel and Statistical Package for Social Scientist (SPSS) computer software packages. The results were then presented using tables, figures and percentages.

RESULTS

Bacteria Growths in Markets Sampled

Table 1 shows the distribution of bacteria growths in the various markets. Kuto Market had the least bacteria growth (17.2%), while Omida and Itoku Markets had the highest (31.0% each).

Table 1: Distribution of Bacteria Growth in Markets

			MARKETS				
			Omida	**Kuto**	**Itoku**	**Lafenwa**	**Total**
Bacterial growth	No growth	Count	12			9	21
		%within Bact. Growth	57.1			42.9	100.0
	Growth	Count	27	15	27	18	87
		%within Bact. Growth	31.0	17.2	31.0	20.7	100.0
Total		Count	27	27	27	27	108
		%within Bact. Growth	25.0	25.0	25.0	25.0	100.0

Bacteria Species on Locations on Fish

The carapace, exoskeleton and entails of smoked prawn harboured equal levels of bacteria. 33.3% each. 93.1% of the bacteria isolated were gram positive while 6.9% were gram negative (Table 2).

Bacteria Species Isolated from Open Markets

Staphylococcus aureus dominated the Omida market having 75.0%. However other bacteria species were sparingly distributed all over the markets (Table 3).

Table 2: Gram's Reaction in Bacteria Growth

			GRAM STAIN		
			Gram Positive	**Gram Negative**	**Total**
Bacterial growth	Growth	Count	81	6	87
		%within Bact. Growth	93.1	6.9	100.0
Total		Count	81	6	87
		%within Bact. Growth	93.1	6.9	100

Table 3: Bacteria Species Isolated from the Markets

		MARKETS				
Bacterial Species		**Omida**	**Kuto**	**Itoku**	**Lafenwa**	**Total**
Staphylococcus. aureus	Count	18			6	24
	%within Bact. spp.	75.0			25.0	100.0
Aerobic Spore Bearer	Count	6	14		10	30
	%within Bact. spp.	20.0	46.7		33.3	100.0
Escherichia coli	Count	3				3
	%within Bact. spp.	100.0				100.0
Gram Positive Cocci	Count			27		27
	%within Bact. spp.			100.0		100.0
Klebsiella spp.	Count				2	2
	%within Bact. spp.				100.0	100.0
Total	Count	27	14	27	18	86
	%within Bact. spp.	31.4	16.3	31.4	20.9	100.0

Table 4: Gram's Reaction of Bacteria Species Isolated

		GRAM STAIN		
Bacterial Species		**Gram Positive**	**Gram Negative**	**Total**
Staphylococcus. aureus	Count	24		24
	%within Bact. spp.	100.0		100.0
Aerobic Spore Bearer	Count	29	1	30
	%within Bact. spp.	96.7	3.3	100.0
Escherichia coli	Count		3	3
	%within Bact. spp.		100.0	100.0
Gram Positive Cocci	Count	27		27
	%within Bact. spp.	100.0		100.0
Klebsiella spp.	Count		2	2
	%within Bact. spp.		100.0	100.0
Total	Count	80	6	86
	%within Bact. spp.	93.0	7.0	100.0

Gram positive organisms accounted for 93.0% of the isolated bacteria species, while the other 7.0% were gram negative organisms (Table 4).

Sensitivity of Bacteria Species to Antibiotics

The susceptibility of the bacteria species to antibiotics are as follows (Tables 5 and 6).

Table 5: Sensitivity of Bacteria Species to Amoxicillin

Bacteria Species		Amoxicillin Resistant
Staphylococcus. aureus	Count	21
	%within Bact. spp.	100.0
Aerobic Spore Bearer	Count	3
	%within Bact. spp.	100.0
Escherichia coli	Count	3
	%within Bact. spp.	100.0
Gram Positive Cocci	Count	3
	%within Bact. spp.	100.0
Klebsiella spp.	Count	2
	%within Bact. spp.	100.0
Total	Count	32
	%within Bact. spp.	100.0

Table 6: Sensitivity of Bacteria Species to Oflaxacin

		OFLAXACIN		
Bacterial Species		**Sensitive**	**Resistant**	**Total**
Staphylococcus. aureus	Count		24	24
	%within Bact. spp.		100.0	100.0
Aerobic Spore Bearer	Count	1	1	2
	%within Bact. spp.	50.0	50.0	100.0
Escherichia coli	Count	3		3
	%within Bact. spp.	100.0		100.0
Gram Positive Cocci	Count		3	3
	%within Bact. spp.		100.0	100.0
Klebsiella spp.	Count	2		2
	%within Bact. spp.	100.0		100.0
Total	Count	6	28	34
	%within Bact. spp.	17.6	82.4	100.0

DISCUSSION

Out of the 108 samples collected from 27 prawn samples bought from the markets in Abeokuta (Lafenwa, Itoku, Kuto and Omida Markets). It was revealed that the prawn samples harboured bacteria growths in the various markets. *Staphylococcus aureus* dominated the Omida market having 75.0%. However other bacteria species were sparingly distributed all over the markets. Gram positive organisms accounted for 93.0% of the isolated bacteria species, while the other 7.0% were gram negative organisms. The following antibiotics were examined for the susceptibility of the bacteria species to them. Amoxilin, Augumentin, Cloxacilin, Erythromycin, Cotrimozazole, Nalaxidic Acid, Nitrofurantin, Oflaxacin, Gentamycin and Tetracycline. However, Gentamycin and Tetracycline were found to be more effective as the bacteria species were more resistant to the other antibiotics. The isolated bacteria species have been implicated in various public health risks such as food poisoning, gastro-enteritis (Hobbs and Roberts, 1993), medical complications, food spoilage and of epidemiological importance.

Samples of locally smoked prawns in open markets in Abeokuta harboured micro-organisms (bacteria), including *Escherichia coli* and *Proteus spp.* with *Micrococcus spp.* and *Bacillus spp.* being the least occurring. While some samples harboured five different types of bacteria. All these micro-organisms are of pathogenic, food

poisoning, food spoilage or even of epidemiological importance (Leitao, 1983). The local method of processing smoke prawn is not very suitable for prolonging the shelf-life of fresh prawn. Packaging of smoked prawn should be fly–proof in order to prevent accelerated spoilage. However, consumers of smoked prawn should subject smoked prawn bought from open markets to further cooking in order to reduce the microbial load to avoid food poisoning.

Methods of handling and processing prawns are generally inadequate and result in major losses, not only in Abeokuta, but Nigeria as a whole. Improvements are needed. Hence, it is vital that emphasis be placed on increased awareness through training for fishermen and processors on improved handling techniques that can result in high quality fish. The use of insulated containers and adequate ice at all stages is required and should be encouraged as this helps maintain the quality of the fish. However, these containers cannot be used without an adequate and cheap supply of ice. Therefore, the provision of cost effective ice production facilities at the major coastal fishing centres should be the government's priority in artisanal fisheries development. Additionally, there is the need to improve education (formal and informal) with regards to the importance of the resource and also the importance of improved processing, storage and distribution. In addition, a grading system could be applied for the smoked prawn where prawns with high grades (high quality) fetch high prices.

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