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Microbial Safety of Smoked Fish -Cat Fish Sold In Navrongo, Upper East Region of Ghana

Patrick Frimpong
Withrow University, Departmentt of Public Health, Box 128, Agona, Ashanti Region, Ghana

Martin Agyei
Ghana Education Service, Diaso Senior High School, Box DW 13, Diaso, Central Region, Ghana

Johnson Mintah-Bonsu
Ghana Health Service, Afrancho Health Centre, Box 3, Kodie, Ashanti Region, Ghana

Abstract
Fish preservation and processing could lead to serious and gross contamination of fish by pathogenic bacteria such as *Salmonella spp, E.coli etc,* which can cause food-borne infections which can be detrimental to human health and sometimes fatal. Smoked fish is defined as a product in which the fish flesh proteins show incomplete coagulation or partially undenatured. Microbial flora distribution in smoked fish products varies largely, depending on the quality of fish at the time of smoking, the smoking temperature and duration, the salt content, and the drying time.Fish is a very good source of protein, vitamins minerals and oil in human diet. It is preferred by many people worldwide because of its high protein value, medicinal value and comparatively highly susceptible to mould, E.coli, faecal coliform contamination. Yet little information is available on the extent of contamination in the region. This study explores the level and extent of contamination of smoked fish in Navrongo, in the Upper East Region in the Northern Ghana. A survey of smoked fish sellers was carried out in the Navrongo market in the Upper East Region of the Northern Ghana. The sample size was taken and a total of ten places of smoked fish sellers were randomly selected to represent the places where samples were collected. Ten samples were taken from these sellers, labeled from A to J and sent to the laboratory for analysis.Samples were prepared by blending and filtering. The samples were grounded and serial dilutions (10^{-1} ─ 10^{-4}) of the homogenized samples were made using sterile distilled water. One millilitre of the serially diluted samples was taken in duplicates and plate count agar was poured at 40°C on the plates. The samples and the medium were properly mixed, allowed to set and incubated at 35°C for 24hrs. The number of colonies on the plates was counted.
Keywords: Microbial Safety, Smoked Fish, Food Safety, Food-borne Infections, fish preservation

1.0 Introduction

Fish is a member of a paraphyletic group of organisms that consists of all gill-bearing aquatic craniates' animals that live all their life in water. Examples of fish species are Cat fish, Herrings, Salmon, Tuna, Cat fish, Mudfish etc (Shaw *et al*., 1977). It is used for a wide range of purposes. Some life fish are used for recreational purposes in aquaria; they may also be used as agents for medicinal purposes and even fish meal for livestock and poultry feed. Research institutions also use them in their academic and research pursuits (Shaw *et al*., 1977).

It is a very good source of protein, vitamins, minerals and oil in human diet. It is preferred by many people worldwide because of its high protein value, medicinal value and comparatively less contamination (Shaw *et al*., 1977). However it is easily contaminated by external pathogens if hygienic and safety conditions are not optimum during the stages involved in cultivation, processing right through to consumption (Lawson *et al.,* 1977).

It is therefore imperative that careful consideration to safety and hygiene is undertaken to prevent contamination during the cultivation/rearing of fish, its management, harvesting, processing, storage and marketing before consumption (Shaw *et al*., 1977). Non compliance to pre-requisite programs (PRPs) and Hazard Analysis and Critical Control Points (HACCPs) in fish preservation and processing could lead to serious and gross contamination of fish by pathogenic bacteria such as *Salmonella spp, E.coli etc,* which can cause food-borne infections which can be detrimental to human health and sometimes fatal (William, 2009).

Smoked fish is defined as a product in which the fish fresh proteins show incomplete coagulation or partially undenatured. Microbial flora distribution in smoked fish products varies largely, depending on the quality of fish at the time of smoking, the smoking temperature and duration, the salt content , and the drying time (Nickelson *et al*., 2001).

Although it is natural to assume that the product being smoked gets hot from exposure to the smoke and is thus cooked, smoking is not always a cooking step due to sometimes the low temperatures involved. In this case smoking alone cannot be used as a method of preventing microbial populating fish. For hard-smoked products with high heat input, relatively heat-stable organisms such as Bacillus, Micrococcus and Yeasts predominate. Additionally, microbial pathogens such as *Listeria monocytogenes*, *Salmonella*, and *Clostridium botulinum* type E may present safety hazards in smoked fish products (Heinitz and Johnson, 1998).

Smoked fish are fish that have been cured by smoking. Foods have been smoked by humans throughout history. Smoking of fish and/or meat products is one of the most ancient processing technologies. It has been for centuries used for preservation, and is still widely used for this purpose among several communities in the third world where up to 70% of the catch is smoked for preservation (Ward, 1995).

Fish and fisheries products are among the most perishable commodities worldwide mainly due to microbial spoilage. About one-third of the world's food production is lost annually as a result of microbial spoilage. In fact, microbial activity is responsible for spoilage of most fresh and of several lightly preserved fish (Lund *et al.*, 2000).

Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella spp.*, and *Clostridium botulinum* (Heintz and Johnson, 1998). Omojowo and Ihuahi, (2006) reported that smoked fish samples from 4 local Markets in Kainji Lake area of Nigeria were dominated by gram-positive bacteria, Potential pathogens, coagulase-positive Staphylococcus, and *Escherichia coli.* Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of smoking and brining. Originally this was done as a preservative (Codex Alimentarius, 1963).

1.1 Background to the Study

In Ghana, like in most sub-Saharan African countries, traditional fishing is practiced in almost all rivers, lakes, ponds and represents an important part of total fish captures. It is an important sector in the national strategies of the fight against poverty. One of the most important producing sites is the water reserve of the Akosombo hydroelectric dam and the Volta Lake and Rivers. Despite the importance of traditional fishing in these areas in Ghana, very few investments are realized in this sector in view of modernizing the activity as well as improving fresh fish preservation. With regard to fresh fish storage, it is estimated that 35% are lost due to the lack of cold chain or proper facilities for preservation (Frimpong *et al.,* 1978).

But local population have developed traditional fish processing techniques that make use of available natural means, namely sun and wood. In this respect, they mainly sun-dry and smoke-dry, more than 75% of fresh fish captured. However this is no panacea to the problem of fish contamination in the country (Shaw, 1977).

Fish processing is usually carried out by women. The method includes fish scaling if necessary, evisceration, washing and draining prior to sun-drying or smoke-drying. For sun-drying, fish are exposed to sun and free air and are turned over from time to time during 48-72hrs period depending on the size of fish and the intensity of sun. Smoking-drying is carried out in terracotta smoking-rooms using various wood species. Fish are smoked for 2-3hrs at 70-80°C, followed by mild smoking (30-35°C) for 24-48 hrs (Krasemann,2009).

But the technology employed by local fishermen is not standardized and most parameters remain uncontrolled. Hence, such essential drying parameters as duration, air humidity and temperature are not precisely determined and mastered. In addition, hygienic conditions of fish capture, processing and storage are questionable. These might have impact on nutritional value and safety of processed fish with possible food toxic-infections. This study is therefore to investigate the effect of traditional fish processing techniques (smoking-drying) on nutritional value and microbiological quality of fish processed, sold and consumed in the Navrongo area. Samples of catfish which are delicacy for the people of Navrongo shall be used in this study. The samples shall be sourced from the local fish smoking places and markets in Navrongo.

1.2 Rational/Justification/Problem Statement

Fish is sterile. Spoilage organisms are introduced during handling, processing, packaging and storage. Processing, preservation and storage of fish in our locality, especially in Navrongo fall short of the ICMSF standards (Codex Alimentarius, 1963).

Due to fungal and bacterial contamination of fish, food-related infections like *salmonella* infections, *E. coli* infections and other fungal infections are prevalent (Codex Alimentarius, 1963). It is against this background that microbial safety of smoked fish sold in Navrongo in the Upper East of Ghana is being studied to collect relevant data's and samples for subsequent laboratory tests to ascertain the types and levels of microbial contamination of fish in the area and to recommend ways to reduce the incidence of microbial contamination of fish.

1.3 Objectives

1.3.1 General objective:

To study the microbial safety of smoked fish sold in Navrongo, Upper East Region of Ghana.

1.3.2 Specific objectives:

1. To determine the microbial load of smoked fish samples.
2. To determine the microbiological quality of smoked fish.

1.4 Scope of Research

Navrongo, the study area is a town in Ghana, near the northern border. It is the capital of Kassena-Nankana District-which is within the Upper East Region of Ghana. Navrongo is the fifty-sixth most populous town in Ghana, with

a population of 27,306 people. Navrongo is an important market town, known for its cathedral and its grotto. Navrongo is located at 10°53′5″N1°5′25″W. The terrain is flat and the ecology is typical of the Sahel – arid grassland with occasional shrubbery (District Assembly, Navrongo, U/E 2012).

The staple food of the people of Navrongo is known as T-Z in English. This is an abbreviation for the Hausa expression *tuo zaafi*, meaning 'very hot'. It is a thick porridge of corn flour eaten by tearing off a chunk and dipping into a soup, usually of okra. This is normally complemented by meat or fish. In the Navrongo area, a large number of people depend on smoked dried fish as sources of protein in their diet. This is due to its ready availability on the local market, its unique flavour and its long shelf life. Smoked fish in Navrongo do not need any specialized storage, thereby making the cost of production lower and a preference for many in the Navrongo area.

However, due to high temperature and high relative humidity in Navrongo (conditions favorable for fungal and bacterial growth) it is well noting the potential of contamination of smoked fish by fungus and or bacteria before, during and after the processes involved in fish smoking in Navrongo. Fungal/bacterial contamination of food is known to affect human health and that of livestock causing health and economic issue in such areas (FDA, 1998).

2.0 Materials and Methods

2.1 Research Design

Cross sectional study was used to select the cat fish. In this study, smoked cat fish samples was purchased and analyzed for levels of microbial contamination. The effect of storage facilities and their possible effect on contamination was assessed. How the storage standards measure up to the ICMSF standards was also assessed.

3.1 Study area

Navrongo, the study area is a town in Ghana, near the northern border. It is the capital of Kassena-Nankana District – which is within the Upper East Region of Ghana. Navrongo is the fifty-sixth most populous town in Ghana, with a population of 27,306 people. Navrongo is an important market town, known for its cathedral and its grotto. Navrongo is located at 10°53′5″N1°5′25″W. The terrain is flat and the ecology is typical of the Sahel – arid grassland with occasional shrubbery. (District Assembly, Navrongo, U/E 2012). It is bordered by the republic of Burkina Faso to the North and Bolgatanga to the East. To the west are Builsa and Sissala Districts and to the south is west Mamprusi district. The district capital is Navrongo. The district occupies a land mass of about 1674km^2,the topography is low lying with an average height of 100m above sea level .The terrain is undulating with isolated hills dotting the landscape .The vegetation of the district is of the Sudan and savannah type with grassland separating deciduous trees.(District Assembly, Navrongo, U/E, 2012) .

3.2 Sampling Methods

- **Sampling (simple random sampling)**

The fish samples were randomly selected section of a larger group; thus a sample of subjects that was randomly selected from a group and is therefore assumed to be representative of that group.

The study was carried out among smoked fish sellers in Navrongo between the period of February and March 2013. It was a cross sectional study involving the collection of smoked fish samples from sellers for laboratory analysis.

Navrongo town has many locations where fish are smoked and sold, out of these ten (10) were randomly sampled. The samples collected were put into sample containers and labeled **A to J.**

TABLE3. 1: Samples for the study and the places of collection

SAMPLES	LOCATION
A	At the main entrance to the Navrongo market
B	Behind SSNIT hostel
C	At the fish mongers market
D	Within the vegetable sellers
E	In front of the mosque inside the market
F	Opposite the main abattoir
G	Within the yam sellers place
H	Opposite the vulcanizing/ fitting shop
I	At the back of the MTN office in the market
J	Behind the fire service office

Before samples will be taken, the **ten** well labeled containers were cleaned with cotton wool containing 70% alcohol. This was done to ensure that bottles that have been already contaminated with any fungi species would be sterilized. The smoked fish samples taken at various sample points/places were taken to the laboratory for analysis.

Smoked cat fish 500g was collected from stalls in Navrongo. The samples were divided into 10 groups according to where they were collected from and labeled accordingly (A-J). The control sample chosen at random was sterilized by autoclave. The samples and control were packed in polythene bags, sealed and kept in paper boxes at ambient (30-33^{o}C) temperature. Samples were subjected to microbial analyses on 0, 1, 2, 3 and 4 weeks of storage.

3.3 Materials (Apparatus and Glassware)

Autoclave (Wet sterilization), Laboratory coat, Measuring cylinder (Graduated 50ml, 250ml)**,** Dispenser, Flat bottomed flask, Micro pipette, Beaker (glass),Funnel Disposable plastic pipettes, Disposable plastic beaker, Test tube, Petri dishes, Stomacher, Paper tape, Sterile swab, Durham's tubes.

Reagents

Distilled water, Peptone water, Isopropyl (50%), Potatoes Dextrose agar (PDA), Blood plasma, Salmonella-Shigella Agar, Eosine Methylene Blue Agar, Buffered peptone water (BWP).

3.4 Methods of sterilization

All glassware were thoroughly washed and rinsed under running tap water. They were finally rinsed again in distilled water. Petri dishes and pipettes were sterilized by heating at 168°c for at least 12 hours in an electrically heated oven.

All inoculating pins, inoculating loops, cork borers were also sterilized by flaming and dipping them in 70% alcohol.

All media were sterilized by autoclaving at 1.05kg\cm^{2} steam pressure (121°c for 15 minutes). Non absorbent cotton wool was used to plug flasks used for autoclaving and aluminum foil was finally used to cover the mouth to prevent the penetration of any condensed water during autoclaving. All inoculations were done under laminar flow in the micro flow chamber.

Procedure

- Sample collection
- Sample preparation
- Examination and recording
- Analysis of results

3.5 Media preparation

Culture media

Salmonella-Shigella Agar

- Salmonella-Shigella Agar was prepared and used for the isolation and identification of Salmonella.

Eosine Methylene Blue Agar

- Eosine Methylene Blue Agar was prepared and used for isolation and identification of E. coli and coliforms.

Cooke's medium

- Cooke's medium was prepared and used for the isolation of fungi from the various smoked cat fish samples. The composition was as follows:
- Agar 12g
- Dextrose 10g
- Peptone 5g
- $Mg\ SO_4.\ 7H_2O$ 0.5g
- $KH_2\ PO_4$ 1.0g
- Rose Bengal 0.035g
- Chloran phenicol 0.05g
- Distilled water 1000ml

Malt extract agar (MEA)

- Malt extract agar (MEA) was prepared and used to obtain pure culture of *Mucor sp*. The composition was as follows:
- Malt Extract 10g
- Agar 2g
- Streptomycin Sulphate 0.05g
- Distilled water 1000ml.

Potato dextrose agar (PDA)

- Potato Dextrose Agar (PDA) was prepared and used to obtain pure cultures of the following fungi; *Aspergillus sp*.
- The Composition was as follows

- Potato 200g
- Agar 12g
- Dextrose 10g
- Streptomycin Sulphate 0.05g
- Distilled water 1000ml

Oxytetracycline glucose yeast extract agar (OGYE)

- OGYE was prepared and used in the estimation of resident fungi on the various samples on the smoked cat fish. The composition was as follows:
- Oxytetracycline Glucose Yeast Extract Agar 37.0g
- Streptomycin sulphate 0.05g
- Distilled water 1000ml

3.6 Microbial analysis

A swab of the skin and smoked cat fish samples were taken with sterile swab stick and 1g representative sample was obtained aseptically from the dorsal muscle of the smoked Cat fish samples. The samples were grounded and serial dilutions (10^{-1} ─ 10^{-4}) of the homogenized samples were made using sterile distilled water.

Total Plate Count (TPC)

This was done using the pour plate method of Harrigan and McCance (1976). One millilitre of the serially diluted samples was taken in duplicates and plate count agar was poured at 40°C on the plates. The samples and the medium were properly mixed, allowed to set and incubated at 35°C for 24hrs. The number of colonies on the plates was counted.

***Salmonella* count**

Samples for detection of salmonella were plated out on Salmonella-Shigella Agar. The plates were incubated at 35^0C for 24hrs. Black colonies showed the presence of *Salmonella sp.*

***Escherichia coli* count**

This was done using Eosine Methylene Blue Agar at 35^0 C for 24hrs. Colonies with green metallic sheen were counted as *E. coli.*

Coliform count

This was also done using Eosine Methylene Blue Agar at 35^0C for 24-48hrs. The samples were first inoculated into lactose broth for 48hrs. The production of gas in the Durham's tubes showed the presence of coliforms. The samples showing gas production were plated out and counted.

Yeast and mould Counts.

This was done by plating out serially diluted samples on Potato Dextrose Agar (PDA) at room temperature (30-35^0 C) for 48-72hrs.

Identification of fungi

This was done using morphological and cultural characteristics according to Smith (1960), Barnett and Hunter (1972), Thom and Raper (1945), Von Arx (1970), Booth (1971) and Samsim and Van Hockstra (1988). Identifications were confirmed by my supervisor.

Laboratory procedure

Faecal coliform

The Most Probable Number (MPN) method was used to determine total and faecal coliforms in the samples. Serial dilutions of 10^{-1} to 10^{-4}were prepared by picking 1 ml of the sample into 9 ml sterile distilled water. One milliliter aliquots from each of the dilutions were inoculated into 5ml of MacConkey Broth with inverted Durham's tube and incubated at 35^0 C for total coliforms and 44^0 C faecal coliforms for 18-24 hours. Tubes showing colour change from purple to yellow and gas collected in the Durham after 24 hours were identified as positive for both total and *faecal coliforms*. Counts per 100 ml were calculated from Most Probable Number (MPN) tables.

E. coli (Thermo tolerant coliform)

From each of the positive tubes identified a drop was transferred into a 5 ml test tube of trypton water and incubated at 44^0 C for 24 hours. A drop of Kovacs' reagent was then added to the tube of trypton water. All tubes showing a red ring colour development after gentle agitation denoted the presence of indole and recorded as presumptive for thermo tolerant coliforms (E. coli). Counts per 100 ml were calculated from Most Probable Number (MPN) tables.

Salmonella

Prepared 10 ml of manufactured formula of Buffered peptone water (BPW) was put in a universal bottle and serial dilution of samples added to it. It is incubated at 37^0 C for 24 hours. Then 0.1 ml of the sample from the BPW is placed in a 10 ml of selenite broth in universal bottle and incubated at 44^0 C for 48 hours. Swaps from the bottle onto SS agar and incubated at 48 hours at 37^0 C. Black colonies on the Salmonella Shigella agar indicate the presence of *salmonella.*

Mould (Fungi)

This was done and enumerated by pour plated method and growth on Potato Dextrose Agar (PDA) serial dilutions

of $10^{-1} - 10^{-4}$were prepared by diluting 1g of the smoked fish into 10ml of sterilized distilled water. One milliliter aliquots from each of the dilutions were inoculated into Petri dishes with already prepared (PDA). The plates were then incubated at 25^0 C for 24 hours.

After incubation all white spot or spread were counted and recorded as mould using colonY counter. In this study, smoked cat fish samples was purchased and analyzed for levels of microbial contamination. The effect of storage facilities and their possible effect on contamination was assessed. How the storage standards measure up to the ICMSF standards wasalso be assessed.

Data was collected on the following;

1. sample smoked fish just after the smoking
2. sample smoked fish at different method of storage
3. Hygiene condition of the selling points before consumption. (Such as very poor, poor, good etc).
4. Hygienic standards of the storage facilities.
5. To carry out laboratory analysis.

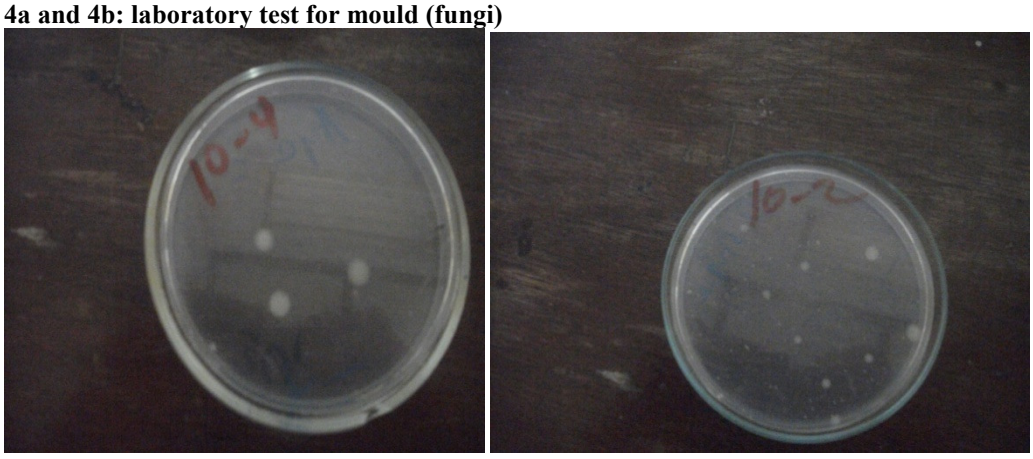
4.0 Analysis of Data

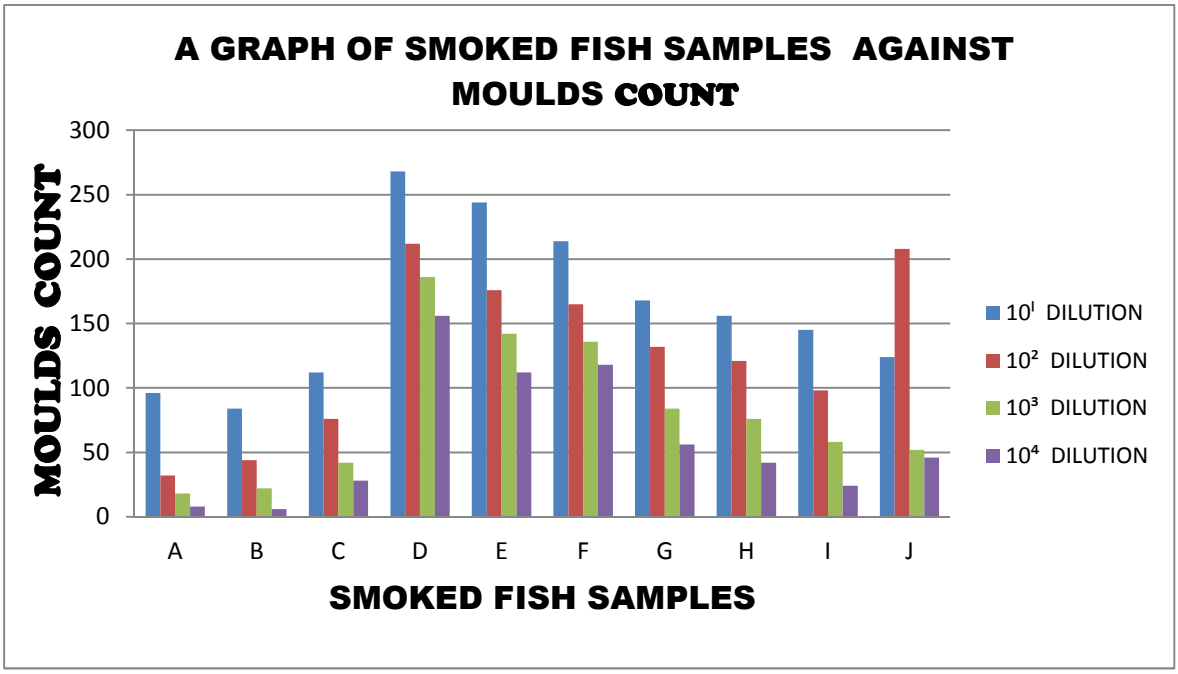
Ten samples were obtained from the Navrongo community for the laboratory analysis each of the samples were run four times to determine the presence if any of moulds (fungi) are found in the dry fish samples from the various locations. The table below shows the Test conducted on the smoked fish samples and the results obtained are as followed.

TABLE 4: TABLE OF VALUES FOR MOULD COUNT (FUNGI) PRESENT IN THE FISH SAMPLES

SMOKED FISH SAMPLES	**MOULD COUNT**				**TOTAL COUNT/ML/CFU**
	SERIES 1 10^{-1}	SERIES2 10^{-2}	SERIES 3 10^{-3}	SERIES4 10^{-4}	
A	96	32	18	8	25540
B	84	44	22	6	21810
C	112	76	42	28	82680
D	268	212	186	156	442470
E	244	176	142	112	320460
F	214	165	136	118	333660
G	168	132	84	56	164720
H	156	121	76	42	127415
I	145	98	58	24	77312
J	124	208	52	46	135415s

4a and 4b: laboratory test for mould (fungi)



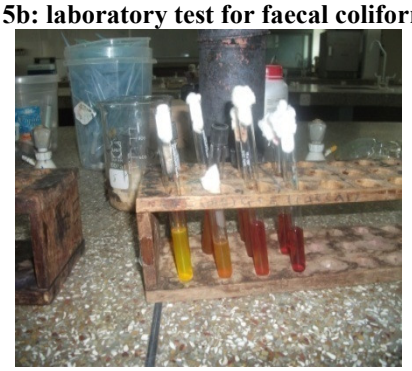


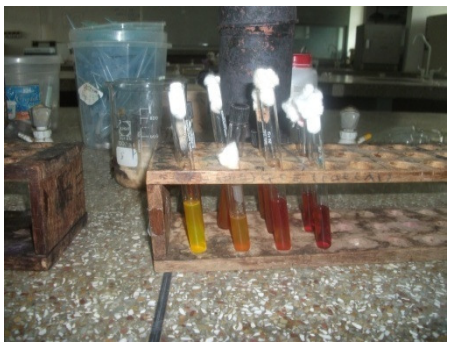
Each of the ten samples was run twice to determine the presence of *E.coli, salmonella* and *faecal coliform* in the smoked fish samples from the various locations. The table below shows the ten tests conducted on the smoked fish samples and the results obtained are as followed.

TABLE 5: TABLE OF VALUES FOR SALMONELA, E-COLI, AND FAECAL COLIFORM COUNT PRESENT IN THE SMOKED FISH SAMPLES

SMOKED FISH SAMPLES	FAECAL COLIFORM CFU/ml		*E.COLI* CFU/ml		SALMONELLA CFU/ml	
A	NIL	NIL	NIL	NIL	NIL	NIL
B	$3.0X10^4$	$3.0X10^4$	NIL	NIL	NIL	NIL
C	NIL	NIL	NIL	NIL	NIL	NIL
D	$9.3 X 10^4$	$9.0 X 10^5$	$9.0 X10^4$	$9.0 X10^4$	NIL	NIL
E	2.3×10^5	2.3×10^5	4.0×10^4	4.0×10^4	NIL	NIL
F	4.3×10^5	4.0×10^5	3.0×10^4	3.0×10^4	NIL	NIL
G	4.3×10^5	4.0×10^5	9.0×10^4	9.0×10^4	NIL	NIL
H	1.5×10^5	1.5×10^5	3.0×10^4	3.0×10^4	NIL	NIL
I	2.3×10^5	2.3×10^5	4.0×10^4	4.0×10^4	NIL	NIL
J	NIL	NIL	NIL	NIL	NIL	NIL

5a and 5b: laboratory test for faecal coliform





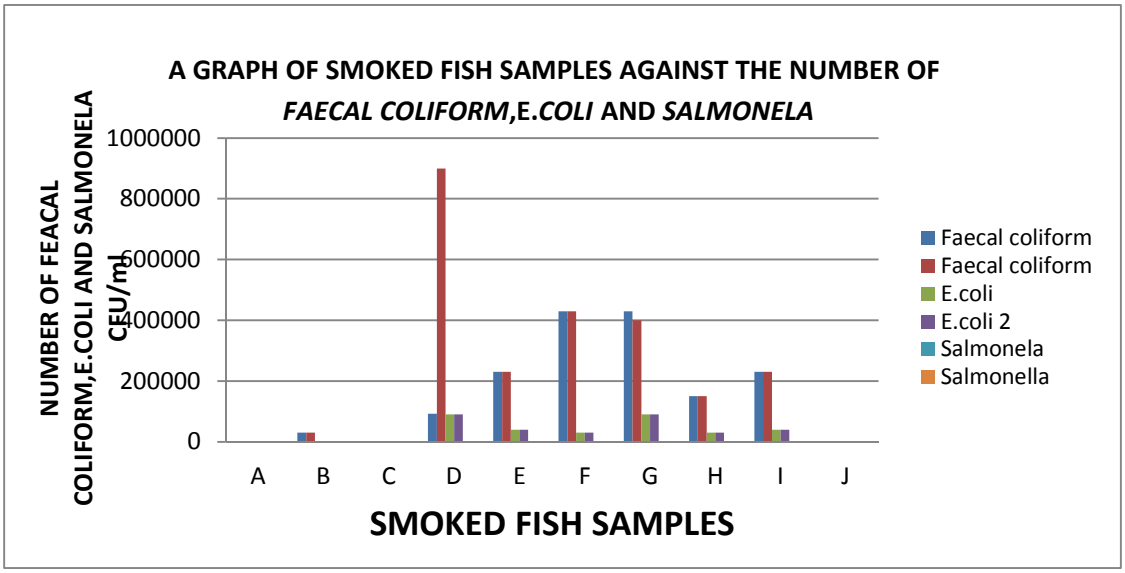


TABLE 6.0: International commission on microbiological specification for food (ICMSF) recommended microbial limits of smoked fish.

	N	c	Bacteria/gram or cm^2	
			m	**M**
salmonella	**5**	**0**	**0**	**-**
E.coli	5 5	3 3	5×10^5 11	10^7 500
Faecal coliform	-	-	-	-
Moulds(fungi)	-	-	-	-

Standard of E. coli 0151:H7.
N = Number of representative sample units.
c = Maximum number of acceptable sample units with bacterial counts between m and M.
m = Maximum recommended bacterial counts for good quality products.
M = Maximum recommended bacterial counts for marginally acceptable quality products.
Plate counts below "m" are considered good quality. Plate counts between "m" and "M" are considered marginally acceptable quality, but can be accepted if the number of samples does not exceed "c." "M" is considered unacceptable quality.
Recommended microbiological limits for *Salmonella* E.*coli*, *faecal coliform* and mould (fungi) in fish and fishery products.
Plate counts below "m" are considered good quality. Plate counts between "m" and "M" are considered marginally acceptable quality, but can be accepted if the number of samples does not exceed "c." Plate counts at or above "M" are considered unacceptable quality (ICMSF, 1986).
Ghana Standard Board acceptable value for E.*coli*= $4x10^1$ CFU/ml
Ghana Standard Board acceptable value *for faecal coliform*= $1x10^5$ CFU/ml

5.0 Discussion of Results

Food poisoning remains a serious problem in developing countries. The presence of E.*coli* could pose danger to consumers. E.*coli* is the major cause of gastroenteritis (Jansson *et al.,* 2008). Processing contributes to fish security by minimizing waste and losses in the food chain and by increasing fish availability and marketability. Fish is also processed in order to improve its quality and safety. Fish safety is a scientific discipline that provides assurance that fish will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. The rapid detection of other microbial contaminants in fish is critical to assess the safety of fish products.

Processing of fish must assure the quality and safety of the final product. Safe fish is one in which physical, chemical or microbiological hazards are present at a level that does not present a public health risk. Safe fish can, therefore, be consumed with the assurance that there are no serious health implications for the consumer.

This test has been designed for use with the procedure and reagents described on the pages above. Do not use materials beyond the expiration date. Deviation from these instructions may not yield optimum result. It is important to obtain a representative sample from a lot. Product should be collected from different locations in a static lot based on a probing pattern. The probe should be draw from the top to the bottom of the lot. The samples obtained from the probes should be grind well and a subsample taken for the test.

A study for the absence and presence of the target food borne pathogens such as *Salmonella, faecal coliform, and E. coli* and fungi (mould) is required to evaluate microbial safety of smoked catfish. The Total Plate Count (TPC) for Coliform, faecal coliform, salmonella count in 100CFU/ml and Fungi (moulds) count in CFU/ml of smoked samples.

Out of the ten samples, 100 tests were conducted. For faecal coliform ten tests were conducted and 10 confirmatory tests was also done to confirm the presence or absence of faecal coliform in the samples. For E.coli 20 tests were done, out of the 20 test conducted 10 were done to confirm the main test. Twenty tests were conducted for the salmonella in which ten was a confirmatory test and the other ten was the main test.

For the salmonella, the level of contamination was zero (0) showing no concentration above the accepted levels of ICMSF standards which for bacterial counts for smoked fish which is zero (0).

From the table of result it is shown that samples A, B, C, and J has no contamination of E.coli at all, representing 40% of the total sample whiles D, E, F, G, H, and I represent 60% of the sample which recorded 9.0×10^4CFU/ml, $4.0\times10^4$100CFU/ml, 3.0×10^4CFU/ml, 9.0×10^4CFU/ml, 3.0×10^4, 4.0×10^4CFU/ml in E.coli count respectively. It could be seen that out of the ten samples presented to the laboratory for analysis the six samples are showing low levels of contamination of E. coli when compared to ICMSF and Ghana Standard Board standard for maximum recommended bacterial counts for good quality products of smoked fish are even low. According to Ghana standard board the action level of E.coli in smoked fish in Ghana is 4 x 10^1CFU/ml, if all things being equal, any value above 4 x10^1 ml/CFU is considered above the action level hence an exposure level of interest. This means that the samples D, E, G and I has high E.coli level in reference to the limit of 4 x 10^1CFU/ml. The E.coli levels of samples A (NIL), B (NIL), C (NIL), F (3.0 X 10^4ml/CFU), H (3.0 X10^4CFU/ml), J (NIL) as obtained from the laboratory analysis was an indication that they were in low concentrations below the accepted standard (4x10^1mlCFU) hence it does not pose any health danger to consumers. Furthermore the E. coli concentrations in the samples D (9.0X104CFU/ml), E (4.0X10^4CFU/ml), G (9.0X10^4CFU/ml), I (4.0X10^4CFU/ml) were found to be above the acceptable standards hence can pose health problems when consumed.

For faecal coliform samples A, C, and J has no contamination. Sample B recorded 3.0×10^4 CFU/ml for both the test and the confirmatory test. In sample D the main test recorded 9.3×10^4 CFU/ml where there was slight increase in the confirmatory test which is 9.0 X 10^5CFU/ml. The main test and the confirmatory test recorded the same values 2.3 X 10^5CFU/ml for E. For F and G there is a slightly decrease in value in the confirmatory test, that is the main test recorded 4.3 X 10^5CFU/ml whiles the confirmatory test recorded 4.0×10^5 for both test respectively. H recorded 1.5 X 10^5CFU/ml for both tests and I also recorded the same values for both tests which are 2.3 X 10^5CFU/ml. It could be seen that out of the ten samples presented to the laboratory for analysis seven of them shows concentrations above the accepted levels of faecal coliform to that of ICMSF and Ghana Standard Board acceptable values. Samples A (NIL), C (NIL), and J (NIL) show levels below that of ICMSF and GSB acceptable standards which is 1 X 10^5ml/CFU hence it does not pose any health danger to consumers. Moreover the faecal concentrations in which the samples B (3.0X104CFU/ml), D (9.3X10^4CFU/ml), E (2.3X10^5CFU/ml), F (4.3 X 10^5CFU/ml), G (4.3X10^5CFU/ml), H (1.5X10^5CFU/ml), and I (2.3X10^5CFU/ml) were found to be above the acceptable standards, hence can pose health problems when consumed.

For the mould (fungi) serial dilution of 10^{-1}—10^{-4} was conducted. In all 40 tests were conducted to show the presence or absence of mould counts in the samples in CFU/ml. In all the ten samples presented to the laboratory for the analysis all of them show some level of contamination with fungi (moulds). In serial dilution 10^{-1} samples C to J recorded the higher level of mould counts of 112CFU/ml, 268CFU/ml, 244CFU/ml, 214CFU/ml, 168CFU/ml, 156CFU/ml, 145CFU/ml, and124CFU/ml respectively in which D recorded the highest number of 268CFU/ml moulds whiles samples A and B recorded a lower mould count of 96CFU/ml and 84ml/CFU respectively in which B recorded the lowest mould count. In 10^{-2} serial dilutions D —H and J recorded the highest number of mould counts of 212CFU/ml, 176CFU/ml, 165CFU/ml, 132CFU/ml, 121CFU/ml, and 208CFU/ml respectively in which D recorded highest mould count of 212CFU/ml whiles the rest A, B C, and I recorded a lower mould count in which B recorded the lowest mould count of 44CFU/ml. D, E and F were the highest mould count of 186CFU/ml, 142CFU/ml, and136CFU/ml in which D has the highest mark of 186CFU/ml and A recorded the lowest mark of 18CFU/ml. Samples A, B, C, G, H, I and J have the lowest mould counts for serial dilution 10^{-3}. D, E and F recorded the highest mould count of 156CFU/ml, 112CFU/ml, and 118CFU/ml in which sample D has the highest mould count of 156. On the other hand A, B, C, G, H, I and J recorded the lowest mould count of 8CFU/ml, 6CFU/ml, 28CFU/ml, 56CFU/ml, 42CFU/ml, 24CFU/ml, and 46CFU/ml respectively in which sample B recorded the lowest mould count of 6CFU/ml in serial dilution 10^{-4}. For sample A the mould counts for 10^{-1}—10^{-4} are 96, 32, 18, and 8 respectively which recorded a total mould count of 2.55 X 10^4CFU/ml.

The total mould counts for the various samples are as follows, 2.5554 X 10^4CFU/ml, 2.181X 10^4CFU/ml,8.268X10^4CFU/ml, 4.424X10^5CFU/ml, 3.2044 X 10^5CFU/ml, 3.3336 X 10^5CFU/ml, 1.6472 X 10^4CFUml, 1.274 X 10^5CFU/ml, 7.731 X 10^4CFU/ml, 1.354 X 10^5CFU/ml from A to J respectively.

The untidy nature of the preparation and sales premises is also a possible source of contamination. From the research it was observed that refuse were left around for some time before they were picked up to throw away.

This could create an environment for flies and these flies could pass pathogens from one food substance to the fish which could lead to contamination. Covering of the fish does not only prevent flies but also, dust from polluting the fish with micro-organisms it carries along with it. The location of some of the fish seller along the roadside could cause some of the vehicles to blow dust into uncovered fish.

Another possible source of contamination might have been improper washing of hands by the sellers and consumers. Washing hands has a significant relationship with the levels of contamination especially with E.coli, implying that there is risk of contamination if seller made direct contact with the fish after visiting the toilet without properly washing the hands with soap.

Personal hygiene and appearance have significant relationship with the levels of contamination, implying that there was risk of contamination if direct contact was made with the smoked fish. It can facilitate the transmission of these pathogens with smoked fish source to humans. The variability in the values obtained is as a result of the origin and places where the fish were smoked and as well as the various conditions under which they were stored.

5.1 Conclusion and Recommendations

From the study fish mongers contribute significantly to the spread of food borne diseases. All samples were found to have mould (fungi) and with the exception of sample A, C and J that do not contain E.coli and faecal coliform, the rest contain E.coli and faecal coliform indicating contaminations from faecal matter and dirty surroundings. The result shows that some of the fish samples have been in the storage place for a longer period and some too have been there for a shorter period indicating the variation in the counts. Fish hygiene practices by the fish mongers are generally low. Most are unaware of the dangers of their poor hygiene attitude hence the continuous sale of contaminated fish to consumers. There are some levels of E.coli, faecal coliform, mould (fungi) in smoked fish sold in Navrongo and moreover the samples were not affected with salmonella.

The fish handling and storage areas should be entirely divorced from:

- Storage of waste materials;
- Storage of packaging materials;
- Storage of cleaning and disinfecting compounds; and
- Storage of wood and wood products used in the smoking process.

Economic impact of faecal coliform, E.coli, mould (fungi)

They have direct effects on human health. Other causes of conjunctivitis in the newborn include E.coli. 40% to 50% of travellers affected with ≥ 3loose stools with enteric symptoms (Alto, 2009).

Food borne illnesses can be fatal as well as cause suffering, discomfort, and debilitation among the survivors. The economic losses from various factors, such as medical treatment, lawsuits, lost wages and productivity, loss of business, recall and destruction of products, and investigation of the outbreaks, can be very high.

5.2 Recommendation

According to the results and the conclusion stated by the researcher the levels of E. coli, faecal coliform and moulds (fungi) in fish sold in Navrongo market can drastically be reduced, if the following food safety measures for smoked fish processing are adhered to:

- ❖ Preventive practices along the length of the fish value chain can help to reduce the risks of E.coli, faecal coliform and mould (fungi) contamination in smoked fish.

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