Original Research Article

Quality Assessment and Evaluation of Bacteria Composition and Abundance in Smoked Fish Processed in Yeji-Pru East District, Ghana.

ABSTRACT

Background: Fish safety and its quality have been a major area of public health concern. This outstanding situation is heavily influenced by several factors ranging from the harvesting environment to the dining table.
Aim: This study aimed in assessing the bacteriological qualities of smoked fish in three (3) major fish processing communities in the Yeji-Pru East District, Ghana.
Study design: The study was a comparative cross-sectional designed to explore information concerning the current status of the phenomenon under consideration, that is bacteria species occurrence and abundance (loads) of smoked fish in Yeji-Pru East District, Ghana. It also was directed towards determining the characteristics of the situation as it exists during the period of the study, from June to December 2017
Method: A closed-ended structured questionnaire was used to collect sociodemographic data from twenty (20) owners of the fish processing site. Forty-eight (48) smoked fish samples including; *Oncorhynchus sp*., *Clupea harengus*, *Chrysichthys auratus* and *Oreochromis niloticus* were surface sterilised, rinsed and dried at 45°C for 24 hours. Ten grams (10g) of each fish sample were diluted with 10 ml of Buffered Peptone Water. Further dilution was prepared using 5 ml of the same diluent. The prepared samples were made to settle for isolations and later inoculated on MacConkey Agar, Shigella-Salmonella Agar, and Salt Mannitol Agar. Microbial colonies were then enumerated using the pour plate method. Data collected were analysed using Microsoft Excel, SPSS and Interactive Chi-square Test.
Results: The results indicated the absence of *Salmonella* and *Shigella* in all fish samples included in the study. The study result also revealed the presence of *Escherichia coli* and *Staphylococcus* species at a rate of 79.2 (n= 38) and 89.6% (n= 43) respectively. The microbial load for *Escherichia coli* and *Staphylococcus* species were community specific. Statistical analysis showed a significant difference ($p < 0.05$) between the microbial loads in fish samples obtained at each study site. However, the mean microbial abundances of each selected sample according to the study site showed no significant difference ($p > 0.05$). The bacteria load observed were lower than the permissible level of human consumption authorised by both the Ghana Standards Authority (GSA) and the International Commission on Microbiological Specifications for Foods (ICMSF).

Conclusion: The study showed that the pre-smoking and post-smoking activities along the fish processing chain could affect the microbial load and diversity. Therefore, the adoption of a good processing practice was highly advocated.

Keywords: *Escherichia coli, Staphylococcus sp, Microbial loads, Ghana Standards Authority (GSA) and International Commission on Microbiological Specifications for Foods (ICMSF).*

ABBREVIATIONS

GSA : Ghana Standard Authority
ICMSF : International Commission on Microbiological Specifications for Foods
HACCP: Hazard Analysis and Critical Control Point
BPW : Buffered Peptone Water
MA : MacConkey Agar
SSA :Shigella-Salmonella Agar (SSA)
SMA :Salt Manitol Agar

1. INTRODUCTION

Fishes are any collection of faunae (animals) that entail all gill-bearing aquatic craniates that lack limbs with distal appendages such as fingers or toes. It forms the majority of cold-blooded aquatic vertebrates [1]. In 2009 it was reported that 40000 species of fish populated the aquatic world [2]. Nutritionally, fish contain a high source of protein, congested with omega-3 fatty acids [3]. It is also made up of food supplements such as vitamin D, calcium, phosphorus, iron, zinc, iodine, magnesium, and potassium [4]. Fish can be eaten fresh or preserved (smoked, fried, or salted) for some time. Fishes form a much-cherished delicacy that cuts across socioeconomic, age, religious, and educational barriers [5]. The Heart Association of America advocates that the consumption of fish twice per week is imperative to boost the human system [6]. The intake of fish has a link with improving the nervous system, reducing diabetes and numerous autoimmune infections [7]. These observations provide some evidence that fish plays a significant role in maintaining and/or boosting the health of humans [8]. Globally, food and water-borne illnesses resulted in 2,200,000 deaths out of a total of one (1) billion reported cases in 2012 [9].

The production of safe fishery products for indigenous consumption in developing nations like Ghana is still a major challenge [10], [11]. Due to this, bacteriological and ecological studies have prompted the need to investigate and determine microbial overloads in fishery products [12], [13]. Also, international organizations such as the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) are working in various ways using varied regulatory mechanisms such as the Hazard Analysis and Critical Control Point (HACCP) and Codex Alimentarius to control the infections associated with food products [14]. The skin, internal organs (especially intestines), and gills of fish get contaminated to varying degrees depending on the water bodies from which they are caught [15]. Fish contamination can occur in an aquatic environment but microbial growth in fish is tamed by the fish's body defence system during life [16]. After death, the defence system breaks down and initiates rapid microbial exponentiation [17]. Freshly caught fish spoils easily and need to be properly preserved to retain its usefulness [18]. Their high moisture and nutrient content make them good substrates for both pathogenic and spoilage microorganisms, which are highly distributed in nature [18]. They can survive and proliferate under various environmental conditions.

There are four (4) techniques used by Ghanaians for preserving fish which include smoking, canning, freezing, and pickling. These techniques are preferred due to longevity, aroma, and protein accessibility [19]. A recent study conducted in Benin City has indicated

some of the specific species of bacteria that contaminate fish. Dominant among these bacteria species are *Proteus mirabilis, Acinetobacter spp*., *Corynebacterium spp., Serratia marcescens, Staphylococcus epidermidis, Streptococcus pyogenes, Klebsiella spp., Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Micrococcus luteus, Enterobacter aerogenes, Flavobacterium spp.* and *Bacillus subtilis* [20].

Therefore, despite the great benefits of smoked fish to humans, it is associated with significant levels of microbial contamination which calls for appropriate measures of handling and preservation conditions [21]. This can be achieved by the enhancement of environmental conditions such as proper fishing and proper fish processing methods. These measures will help to improve the microbial worth of smoked fish [22], [23]. Nevertheless, there are no data on the microbial occurrence and loads of smoked fish processed in Yeji-Pru East, Parambo, and Prang. There is therefore imperative to investigate the occurrence, distribution, and bacteriological abundance in smoked fish. The study also established the link between the fish processing activity and its impact on the bacteriological quality of smoked fish processed in the Yeji-Pru East District.

2. MATERIALS AND METHODS

2.1 Study design

The study was a comparative cross-sectional designed to explore information concerning the current status of the phenomenon under consideration, that is bacteria species occurrence and abundance (loads) of smoked fish in Yeji-Pru East District, Ghana. It also was directed towards determining the characteristics of the situation as it exists during the period of the study, from June to December 2017

2.2 Variables of Interest

Bacteria occurrence and abundance (loads) in smoked fish processed in Yeji

2.3 Study site

The study was carried out at the Yeji Konkoma, Yeji Kou and Yeji Nsuoano, located in different geographical areas in the Yeji-Pru East District of Ghana. Geographically, Yeji lies 8°13′N 0°39′W within Pru-District which lies between Longitudes 0°30°W and 1°26°W and Latitudes 7°50°N and 8°22°N. Bacteriological analysis was conducted at the Department of Biological Science, Kwame Nkrumah University of Science and Technology (KNUST) located in Kumasi, Ashanti region of Ghana.

2.4 Sample size and sampling procedure

A simple random sampling technique was used to select all the forty-eight smoked fish species for bacteriological analyses. Sixteen (16) smoked fish species were randomly sampled from each of the three study areas (Yeji Kou, Yeji Konkoma and Yeji Nsuoano) in the periods of June and December 2017. The samples were placed in germ-free plastic bags and labelled appropriately based on the sampling area (Yeji Kou, Yeji Konkoma and Yeji Nsuoano) and transported to the Department of Biological Science, Kwame Nkrumah University of Science and Technology (KNUST) located in Kumasi, Ashanti region of Ghana for bacteriological analyses.

2.5 Preparation and sterilisation of media

The fish species sampled were surface sterilised separately in 3.5% sodium hypochlorite solution (w/v) with constant agitation for 7 minutes and rinsed thoroughly with sterile distilled water until the traces of hypochlorite were removed. They were then dried in an oven at 45°C for 24 hours. The media obtained from Oxoid Limited, England were also

prepared in sterile condition per the manufacturer's directives. Sterility checks of each media and diluent were done by incubating them overnight at their respective temperatures for the required time.

2.6 Inoculation and counting of bacteria colonies

Samples of the various body parts of the fish were collected separately under aseptic conditions. An amount of 10g of each sample was added to 10 ml of Buffered Peptone Water (BPW) to prepare an initial dilution (stock solution) and serial dilutions 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were prepared and used as diluent. Ten grams (10g) of each sample of smoked fish was weighed and placed into ninety millilitres (90mls) of sterilised distilled water and placed in a purifier for fifteen seconds (15 sec). One millilitre (1ml) of aliquots from each of the dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were inoculated into a Petri dish with already prepared agar. MacConkey Agar (MA), Shigella-Salmonella Agar (SSA), and Salt Manitol Agar (SMA) were used. An antiseptic pipette was used to transfer one (1) millilitre of aliquots from each dilution of the test sample and labelled appropriately. The prepared samples were later made to settle and the various bacteriological microbes were isolated. This was done by carefully streaking the inoculums on their agar to enhance uniform distribution on the agar. After the inoculation and streaking, they were incubated at their specified temperature. The colonies of bacteriological microbes were enumerated using the pour plate method on a colony counter.

2.7 Enumeration of bacteria abundance

The Total Plate Count (TPC) of bacteria on smoked fish sampled from the selected study areas namely, Yeji Kou, Yeji Konkoma and Yeji Nsuoano, were in values of respective serial dilution factors of 10^{-1} (1:10), 10^{-2} (1:100), 10^{-3} (1:1000) and 10^{-4} (1:10000). After the values were obtained, their colony forming unit and mean total counts colony forming unit (cfu/ml) were also estimated using the notations:

$$Colony\ forming\ unit\ \left(\frac{cfu}{ml}\right) = \frac{colonies\ formed}{volume\ of\ culture\ plate \times dilution\ factor}$$

$$Mean\ total\ count\ \left(\frac{cfu}{ml}\right) = \frac{(colonies\ )}{Volume\ of\ culture\ plate}$$

2.8 Quality Control

Quality control measures were done during the analysis to confirm the accuracy of the results. In every analytical batch, all samples were analysed separately with a series of serial dilutions to ensure accuracy.

2.9 Data Processing and Analysis

Data collected in the current study were analysed using Microsoft Excel version 2016 (Microsoft, USA), Software Package for Social Sciences (SPSS) version 23 (IBM, USA), Interactive chi-square calculator (Preacher, K. J. 2001) and MINITAB version 18 computer software (UK). The test significance chosen was 5%.

3.0 RESULT

3.1 Socio-cultural study of fish mongers

The result of the study revealed that all the fishmongers were between the age of 25 to 54 years and a mean age of 44 years. The majority of the fishmongers were skilled permanent workers, married with basic educational background. The mongers employed various pre-smoking and post-smoking activities to increase the shelf life of fish. However,

some level of unhygienic pre-smoking and post-smoking practices among the mongers were observed. All the studied mongers' transport fish from landing sites using tricycles before smoking is done. About 80% (n= 16) of the mongers indicated that the time taken to transport fresh fish from landing sites to various homes for smoking ranged from ≤10 minutes to 20 minutes. The overall mean time for fish to be transported from the landing sites to the smoking sites was 14 (95% CI: 10.54 - 17.36) minutes. None of the fishmongers used chemicals before and after smoking their fish. The time taken for the mongers to complete smoking ranged from 4 hours to 9 hours before packaging. The majority of the mongers 35% (n=7) used both cylindrical and rectangular mud ovens. Furthermore, an equal proportion of 20% (n=4) used the cylindrical metal oven and 20% (n=4) used rectangular mud ovens. Also, 10% (n=2) used both cylindrical mud ovens and cylindrical metal ovens whereas 10% (n=2) used both cylindrical mud ovens and rectangular mud ovens. Finally, only one (1) of the studied participants employed the use cylindrical mud oven.

Table 1: Distribution of fish mongers stratified by socio-cultural variables

Socio-cultural variable	Frequency N=20	Percent (%)
Residence		
Yeji Nsuoano	7	35
Yeji Konkoma	6	30
Yeji Kou	7	35
Education		
SHS Leavers	4	20
Basic School leavers	16	80
Marital Status		
Married	18	90
Unmarried	2	10
Skill		
Skilled	19	95
Semi-skilled	1	5
Unskilled	0	0
Nature of Job		
Permanent	20	100
Casual	0	0
Methods of Harvesting		
Netting	14	70
Both netting and Trapping	6	30
Time Taken for Transportation of Fish to Be Smoked		
Up to 10 minutes	8	40
11-20 minutes	8	40
21-30 minutes	4	20
Mode of Transportation		
Tricycle	12	60
Truck	2	10
Vehicle	6	30
Ovens Used for Fish Smoking		

Cylindrical mud oven	1	5
Both cylindrical mud ovens and cylindrical metal oven	2	10
Both cylindrical mud ovens and rectangular mud ovens	2	10
Cylindrical metal oven	4	20
Both cylindrical mud ovens and rectangular mud ovens	7	35
Rectangular mud oven	4	20

3.2 Occurrence and composition of bacteria species on smoked fish in Yeji-Pru east district

The study revealed the absence of *Salmonella* and *Shigella* and the presence of *Escherichia coli* and *Staphylococcus* species on 89.6% (n= 43) of smoked fish species as shown in **Figure 1**. The interactive Chi-Square of goodness-of-fit showed a spatial significant difference between smoked fish species that tested positive for microbial occurrence and smoked fish species that tested negative for microbial occurrence (χ^2 =30.083, DF = 1, *p*-value < 0.05). Approximately 79.2% (n= 38) of the fishes were contaminated with *Escherichia coli* and 89.6% (n= 43) were contaminated with *Staphylococcus* sp. Approximately 79.2% (n= 38) of the fishes were contaminated with *Escherichia coli* and 89.6% (n= 43) were contaminated with *Staphylococcus* species.
Also, out of the four categories of fish tested for bacteria contamination, all the salmon had *E. coli*. However, *E. coli* contamination rates of herring, tilapia and catfish were 50.0%, 75.0% and 91.7% respectively. All tilapia and catfish species examined were contaminated with Staphylococcus species whereas Staphylococcus species contamination rates of salmon and herring were 75% and 83.3% respectively as shown in **Table 2.**

Figure 1. Bacteria Count for Smoked Fish

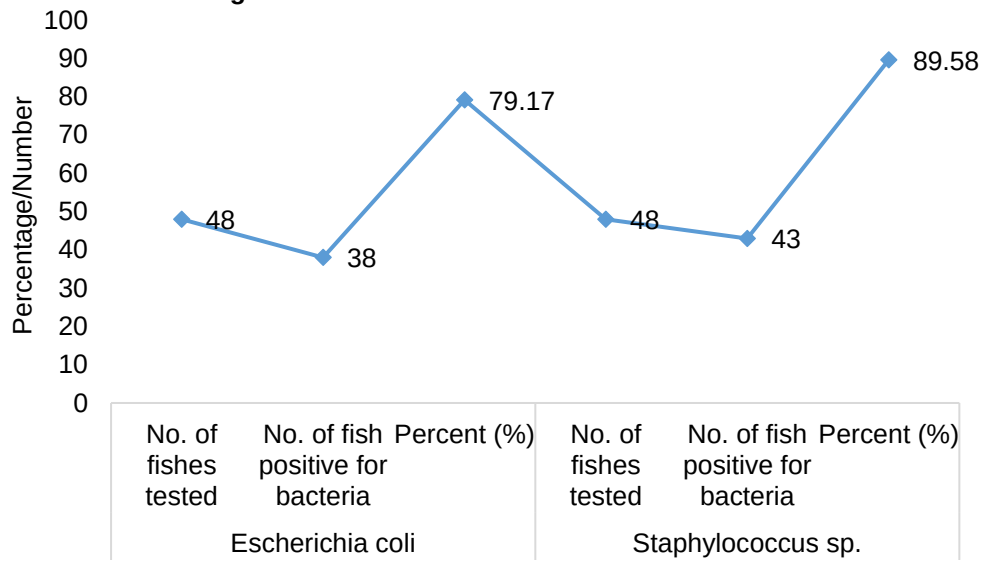

Table 2: Number of Fishes Sampled, Number of Fishes That Were Contaminated with *E. Coli* and *Staphylococcus sp*. At Yeji

	Fish	Number sampled	Number infected	Per cent (%)
E. coli	Salmon	12	12	100.00
	Herring	12	6	50.00
	Catfish	12	11	91.67
	Tilapia	12	9	75.00
Staphylococcus sp.	Salmon	12	9	75.00
	Herring	12	10	83.33
	Catfish	12	12	100.00
	Tilapia	12	12	100.00

3.3 Microbial Abundance of *Escherichia coli*

Escherichia coli composition differed according to fish species as indicated in **Tables 3 and 4**. Pacific salmon (*Oncorhynchus sp*.) had *Escherichia coli* total abundance of 1.1×10^{6}*cfu/ml* [4.2×10^{5} for Yeji Kou, 3.0×10^{5} for Yeji Konkoma and 3.9×10^{5} for Yeji Nsuoano] with 262 *cfu/ml* average number of colonies formed [99 *cfu/ml* for Yeji Kou, 78 *cfu/ml* for Yeji Konkoma and 85 *cfu/ml* for Yeji Nsuoano]. Also, *Escherichia coli* abundance on herrings (*Clupea harengus*) sampled from all the selected study areas was 9.7×10^{3}*cfu/ml* [2.9×10^{3}*cfu/ml* for Yeji Kou, 3.7×10^{3}*cfu/ml* for Yeji Konkoma and 3.1×10^{3}*cfu/ml* for Yeji Nsuoano] with the 21 *cfu/ml* as the overall average number of colonies formed [6 *cfu/ml* for Yeji Kou, 8 *cfu/ml* for Yeji Konkoma and 7 *cfu/ml* for Yeji Nsuoano]. Moreover, *Escherichia coli* on catfish (*Chrysichthys auratus*) sampled from the studied areas was 8.7×10^{5}*cfu/ml* [3.7×10^{5}*cfu/ml* for Yeji Kou, 7.5×10^{4}*cfu/ml* for Yeji Konkoma and 4.3×10^{5}*cfu/ml* for Yeji Nsuoano] and also had the overall average number of colonies formed to be 159 *cfu/ml* [53 *cfu/ml* for Yeji Kou, 44 *cfu/ml* for Yeji Konkoma and 63 *cfu/ml* for Yeji Nsuoano]. Finally, *Escherichia coli* on tilapia species sampled from all the selected study areas was 2.0×10^{5}*cfu/ml* [5.0×10^{4} for Yeji Kou, 1.1×10^{5} for Yeji Konkoma and 4.4×10^{4} for Yeji Nsuoano] with 123 *cfu/ml* being the overall average number of colonies formed [37 *cfu/ml* for Yeji Kou, 47 *cfu/ml* for Yeji Konkoma and 40 *cfu/ml* for Yeji Nsuoano]. There were no considerable spatial variations between the average number of colonies formed in all study areas ($p > 0.05$).

Table 3: Colony Forming Units of *Escherichia Coli*

Sample	Site	Abundance (*cfu/ml*)			
Salmon	Yeji Kou	6.1×10^{3}	2.4×10^{4}	1.1×10^{5}	2.8×10^{5}
	Yeji Konkoma	4.8×10^{3}	1.8×10^{4}	9.7×10^{4}	1.8×10^{5}
	Yeji Nsuoano	4.7×10^{3}	2.6×10^{4}	8.8×10^{4}	2.8×10^{5}
Herrings	Yeji Kou	3.8×10^{2}	2.5×10^{3}	0.0×10^{0}	0.0×10^{0}
	Yeji Konkoma	4.8×10^{2}	3.3×10^{3}	0.0×10^{0}	0.0×10^{0}
	Yeji Nsuoano	3.8×10^{2}	2.8×10^{3}	0.0×10^{0}	0.0×10^{0}
Catfish	Yeji Kou	2.3×10^{3}	1.5×10^{4}	1.2×10^{5}	2.3×10^{5}
	Yeji Konkoma	2.3×10^{3}	1.5×10^{4}	5.8×10^{4}	0.0×10^{0}
	Yeji Nsuoano	2.7×10^{3}	1.9×10^{4}	1.3×10^{5}	2.8×10^{5}
Tilapia	Yeji Kou	2.3×10^{3}	1.0×10^{4}	3.8×10^{4}	0.0×10^{0}
	Yeji Konkoma	2.4×10^{3}	1.4×10^{4}	9.0×10^{4}	0.0×10^{0}
	Yeji Nsuoano	2.4×10^{3}	1.4×10^{4}	2.8×10^{4}	0.0×10^{0}

Table 4: Interactive Chi-square Test for Goodness-of-fit on the average number of *Escherichia coli* colonies formed.

Fish species	Observed cfu/ml	Expected cfu/ml	$\chi2$	DF	*p*
Salmon	99 78	87.33	2.618	2	>0.27

	85				
Total	262				
Herring	6	7.00	0.286	2	>0.866
	8				
	7				
Total	21				
Catfish	53	53.33	3.388	2	>0.184
	44				
	63				
Total	160				
Tilapia	37	41.33	1.274	2	>0.529
	47				
	40				
Total	124				

3.4 Microbial Abundance of *Staphylococcus species*

Approximately all sampled smoked fish species had colony-forming units of *Staphylococcus* species, even though their loads varied according to fish species as shown in **Tables 5 and 6**. *Staphylococcus* species loads on pacific salmon [*Oncorhynchus sp*.] sampled from all the selected study areas was 1.9×10^5*cfu/ml* [6.9×10^4 for Yeji Kou, 4.2×10^4 for Yeji Konkoma and 7.9×10^4 for Yeji Nsuoano] with the overall average number of colonies formed being 175 *cfu/ml* [61 *cfu/ml* for Yeji Kou, 50 *cfu/ml* for Yeji Konkoma and 65 *cfu/ml* for Yeji Nsuoano]. Also, the total load of *Staphylococcus spp.* on sampled herring species from all the selected study areas was 7.0×10^5*cfu/ml* [1.5×10^5 for Yeji Kou, 4.3×10^5 for Yeji Konkoma and 1.2×10^5 for Yeji Nsuoano] and had 537 *cfu/ml* overall average number of colonies formed [179 *cfu/ml* for Yeji Kou, 184 *cfu/ml* for Yeji Konkoma and 174 *cfu/ml* for Yeji Nsuoano]. Furthermore, the total abundance of *Staphylococcus spp.* on catfish species sampled from all the selected study areas was 2.2×10^6*cfu/ml* [8.9×10^5 for Yeji Kou, 3.3×10^5 for Yeji Konkoma and 9.6×10^5 for Yeji Nsuoano], forming 385 *cfu/ml* overall average number of colonies [147 *cfu/ml* for Yeji Kou, 104 *cfu/ml* for Yeji Konkoma and 132 *cfu/ml* for Yeji Nsuoano]. Finally, tilapia species sampled from all the selected study areas had a total abundance of 1.2×10^6*cfu/ml* [3.4×10^5 for Yeji Kou, 3.4×10^5 for Yeji Konkoma and 5.2×10^5 for Yeji Nsuoano] *Staphylococcus spp.* with the overall average number of colonies formed been 273 *cfu/ml* [92 *cfu/ml* for Yeji Kou, 78 *cfu/ml* for Yeji Konkoma and 103 *cfu/ml* for Yeji Nsuoano. There were no considerable spatial variations between the average number of colonies formed in all study areas ($p > 0.05$).

Table 5: Colony Forming Units of *Staphylococcus sp.*

Sample	Site	Abundance (*cfu/ml*)			
Salmon	Yeji Kou	4.1×10^3	1.5×10^4	5.0×10^4	0.0×10^0
	Yeji Konkoma	3.6×10^3	1.1×10^4	2.8×10^4	0.0×10^0
	Yeji Nsuoano	4.4×10^3	1.5×10^4	6.0×10^4	0.0×10^0
Herrings	Yeji Kou	1.4×10^4	2.4×10^4	1.2×10^5	0.0×10^0
	Yeji Konkoma	1.4×10^4	2.5×10^4	1.4×10^5	2.5×10^5
	Yeji Nsuoano	1.4×10^4	2.2×10^4	8.0×10^4	0.0×10^0
Catfish	Yeji Kou	6.0×10^3	6.1×10^4	2.0×10^5	6.3×10^5
	Yeji Konkoma	5.6×10^3	3.4×10^4	1.2×10^5	1.8×10^5
	Yeji Nsuoano	6.2×10^3	4.1×10^4	2.1×10^5	7.0×10^5
Tilapia	Yeji Kou	5.2×10^3	2.4×10^4	1.5×10^5	2.0×10^5
	Yeji Konkoma	5.0×10^3	1.6×10^4	9.0×10^4	2.3×10^5
	Yeji Nsuoano	5.4×10^3	3.0×10^4	1.6×10^5	3.3×10^5

Table 6: Interactive Chi-square Test for Goodness-of-fit on the average number of *Staphylococcus spp*. colony formed.

Smoked fish species	Observed cfu/ml	Expected cfu/ml	χ2	DF	*p*
Salmon	61 50 65	58.66667	2.057	2	>0.358
Total	176				
Herring	179 184 174	179	0.279	2	>0.867
Total	537				
Catfish	147 104 132	127.6667	7.462	2	<0.024
Total	383				
Tilapia	92 78 103	91	3.451	2	>0.178
Total	273				

3.5 Comparing Bacteria Abundance of Smoked Fish Species Sampled with The Specification Level of Ghana Standards Authority (GSA) And International Commission on Microbiological Specifications for Foods (ICMSF)

Table 7: Comparing The *E.Coli* Abundance (Loads) With GSA/ICMFS Permissible Level

Smoked fish species	Total load	GSA/ICMSF permissible level	χ2	DF	*P*
Salmon	1100000	10000000	7136036	1	0.000
Herring	9700	10000000	9970938	1	0.000
Catfish	870000	10000000	7668528	1	0.000
Tilapia	200000	10000000	9415686	1	0.000

Table 8: Comparing the *Staphylococcus spp.* Abundance (loads) with GSA/ICMFS permissible level

Smoked fish species	Total load	GSA/ICMSF permissible level	χ2	DF	*P*
Salmon	190000	10000000	9444171	1	0.000
Herring	700000	10000000	8083178	1	0.000
Catfish	2200000	10000000	4986885	1	0.000
Tilapia	1200000	10000000	6914286	1	0.000

4.0 DISCUSSION

Before fish were smoked, various pre-smoking and post-smoking activities were employed. The various pre-smoking activities as done in the current study areas were found to require several materials. Those observed in the current study consisted of knives, metallic or plastic pans, cutting media (boards or wood) and water (brine or fresh). It was also observed that descaling and general cutting were done on unhygienic wooden surfaces, packaging boxes and metallic sheets. These observations were peculiar in all the study areas irrespective of the improvement in material usage and the cleanliness of smoking sites. Rinsing of fishes to be smoked were done using water fetched from the Volta Lake.

The mongers used the same water that they fetched at the beginning of the rinsing activity to wash all the batches of fish that they got throughout the day. This was irrespective of the bloodiness and cloudiness of the rinsing water. They only discarded the water after the overall completion of the pre-smoking process. Since mongers studied displayed their processed fishes plainly without covering them, it exposed the fishes to be cooled to flies and other carriers of infections like the air. These activities along the processing chain could bring about bacterial infections in the form of cross contaminations. For example, contaminants such as *Campylobacter*, *Escherichia coli*, *Staphylococcus spp. Vibrio*, *Shigella*, *Salmonella* and *Pseudomonas* can last on surfaces of kitchen materials (including cutting media and knives) and packaging boxes for longer periods [24], [25]. This is in agreement with a study conducted by [26] which indicated that bacteria can be transferred from processing materials onto prepared meals.

The majority of fishery products are passive hosts of *Salmonella* and *Shigella* species even though these microbial contaminants are commonly waterborne [27]. Fish species serving as passive hosts are apparently due to injuries on the part of the fish species or environmental stress such as high temperature, poor quality and faecal contamination of water from where fishes are harvested. Although earlier studies showed that the water of Lake Volta was contaminated with microbes [28], no colonies of both Salmonella and Shigella species were isolated in smoked fish species processed using water from Lake Volta for washing or rinsing. This finding was in line with earlier reports which did not find Salmonella sp. and Shigella on smoked fish species [29], [30].

Contamination of fish species by *Escherichia coli* mostly results from the unhygienic condition of the smoking environment and materials used along the fish processing chain at large [31], [32]. The hygienic conditions of the fish-smoking environment in the selected study sites were commonly unclean. Concerning this, there is a higher prospect of cross-contamination from the processing environment onto processed fish species. For example, at Yeji Nsuoano waste bins used for collecting unwanted remains of fish parts were left uncovered at the fish smoking site. Thus, there were a lot of housefly populations at the processing site. Houseflies have been evaluated through a series of research as vectors of several foodborne pathogens and an example is *Escherichia coli* [33]. Also, in Yeji Konkoma and Yeji Kou, the boxes used as packaging media for some species of fish were left in the open near the processing site and this probably exposed the smoked fish species to contaminants which were probably present on the packaging boxes. It was also noted that these same packing boxes were used as spread layers to prevent the smoked fish from touching the ground. Moreover, the water obtained from the Volta Lake was used for rinsing fish to be smoked. Due to various anthropogenic activities such as sewage disposal, defecation and droppings of farm animals along the shore and even into the Lake as reported by the study conducted by Clottey et al*.,* 2016, fish from the lake may be contaminated. The study by [34] on the quality of Volta Lake also demonstrated that the lake is being significantly contaminated with microbes. This possibly contributed to the cause of the *Escherichia coli* contamination of the smoked fish species as observed in the current study. The observation from this current study is also in agreement with earlier studies that revealed Escherichia coli in smoked fish species processed in Ghana [23], [28], [35], [36].

Staphylococcus species are commonly found in the nose, mouth and on the skin of humans and animals. Contamination of foods by *Staphylococcus spp.* often result from poor handling. People who carry *Staphylococcus spp.* can contaminate food by merely touching it [37]. It was previously reported that the toxins produced by Staphylococcal contaminants are also resistant to heat, this is an indication that simple heat may destroy these microbial contaminants[38], [39]. As part of observations made in the current study, the majority of the studied fish mongers used their bare hands to undertake all activities (including both pre-smoking and post-smoking) associated with fish processing. This brings about skin contact, which may initiate staphylococcal contamination of smoked fish species. These findings were in agreement with earlier reports in Nigeria which indicated the occurrence of Staphylococcus species on samples of fresh and smoked fish species

from Benin City, Minna Metropolis (Niger State) and Benin metropolis [39], [40]. This finding was in line with other studies which detected Staphylococcus species on smoked fish from various markets in Ghana [23], [28], [35], [36].

5.0 CONCLUSION

Bacteria abundance on sampled smoked fish species used in the current study was high and the reason associated with these high counts is discussed in the previous section. To create awareness among the public on bacteriological and smoked fish-based food safety, the abundances observed in the study were compared with the permissible levels of the Ghana Standards Authority (GSA) and the International Commission on Microbiological Specifications for Foods (ICMSF). The level of specification is 1.0×10^7 *cfu/g*. From Tables 7 and 8, there are statistically significant differences between the observed bacteria load and the permissible level of the Ghana Standards Authority (GSA) and the International Commission on Microbiological Specifications for Foods (ICMSF) ($p<0.05$). As a result, all bacteria loads observed were classified as low when compared with the permissible levels of Ghana Standards Authority (GSA) and International Commission on Microbiological Specifications for Foods (ICMSF) 1×107 cfu/g or cfu/ml. That is the enumerated bacteria abundances on sampled smoked fish species in each of the studied sites differed significantly ($p<0.05$) when compared with the permissible standards. Nevertheless, the microbial counts could be decreased significantly through proper pre-smoking and post-smoking activities.

CONSENT AND ETHICS APPROVAL

A detailed plan of the study and objectives were submitted to the Head of the Department of Science Education who gave his approval first, and then the project supervisor assessed it. The proposal was submitted to the Yeji Pru District Fishery Department before the study was undertaken. Permission was also sought from the chief market women and fishmongers of each study site. The study was conducted under all applicable laws and regulations. Participants were informed that the information collected from them would only be utilised by stakeholders to make policy decisions. Additionally, participants were informed that their participation in the study was voluntary, that there were no known dangers, and that they had the right to withdraw at any time. The results were not provided along with any personally identifiable information, such as participant names taken from the surveys. Concerns were also sought from the designers and users of the various kiln (oven) types before the images of the kilns were taken.

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