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Evaluation of Bacteria Composition in Smoked Fish Processed in Yeji-Pru East District, Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Author DB carried out the study's methodology and concepts. The formal analysis was conducted by authors DB, JG, DH, and IB who also oversaw the literature searches. The first draft of the paper was written by authors DB and JG. The work was read and modified by the authors DH and DB. All authors read and approved the final manuscript.

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ABSTRACT

Background: Fish safety and quality have been a major area of public health concern. This situation was heavily influenced by several factors ranging from the harvesting environment to the dining table.
Aim: This study aimed at assessing the bacteriological quality of smoked fish in three (3) major fish processing communities in the Yeji-Pru East District, Ghana.

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Study design: The study was a comparative cross-sectional design to explore 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 existed during the period of the study, from June to December 2017.
Method: A closed-ended structured questionnaire was used to collect sociodemographic data from 20 owners of the fish processing site. Forty-eight 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 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 at a test significance of 5%.
Results: The results showed absence of *Salmonella* and *Shigella* in all fish samples studied. However, it 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 *E. 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, there was no spatial variation in the mean microbial abundances of each selected sample ($p > 0.05$). The bacteria load observed were lower than the permissible level for human consumption authorised by both the Ghana Standards Authority and the International Commission on Microbiological Specifications for Foods.
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. They form the majority of cold-blooded aquatic vertebrates [1]. In 2009 it was reported that 40000 species of fish populated the aquatic world [2]. Fishes form a much-cherished delicacy that cuts across socioeconomic, age, religious, and educational barriers [3]. Fish can be eaten fresh or preserved (smoked, fried, or salted) for some time. Nutritionally, fish contain a high source of protein, congested with omega-3 fatty acids [4]. It is also made up of food supplements such as vitamin D, calcium, phosphorus, iron, zinc, iodine, magnesium, and potassium [5]. The Heart Association of America advocates that the consumption of fish twice per week is imperative to boost the immune 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].

Currently, 7.8 billion people are living in the world, and 56 million people die yearly. Of the 56 million estimated yearly death, 7.5% was a result of foodborne infections. Etiological agents implicated in these are *Campylobacter* and *Listeria monocytogenes* [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.

1.2 Statement of Problem

Consumption of fishery products has intensified in modern days. Ghana's average per capita consumption of fish is estimated around 24.2 to 26 kg [19]. This exceeds the world's average consumption of 13kg. Local consumption accounts for around 60% of global animal protein consumption and is expected to account for 80% of domestic fish production [19]. This is because of the preference for fish protein which is of low cholesterol. Despite the great benefits of fish to humans, it is associated with significant levels of microbial contamination which calls for appropriate measures of handling and preservation conditions [20]. This can be achieved by the enhancement of environmental conditions such as proper fishing and proper fish processing and preservation methods. These measures will help to improve the microbial worth of smoked fish [21,22]. There are four (4) techniques used by Ghanaians for preserving fish. These are smoking, canning, freezing, and pickling. These techniques are preferred due to longevity, aroma, and protein accessibility [23].

Each year, contaminated food contributes to 420 000 fatalities and 600 million cases of foodborne illness worldwide. Children under 5 years old make up 30% of foodborne mortality victims [24]. 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* [25]. Nevertheless, there was no available data on the microbial occurrence and loads of smoked fish processed in Yeji-Pru East District, Ghana. It is, therefore, imperative to investigate the occurrence, distribution, and bacteriological abundance of smoked fish processed and sold on the open market of Yeji, Pru East District, Ghana. This study aimed at assessing the bacteriological quality of smoked fish in three (3) major fish processing communities in the Yeji-Pru East District, Ghana. 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.

1.3 Justification

Considering the consequences of bacteria such as cholera, shigellosis, typhoid fever, severe dysentery and other gastrointestinal infections to humans, it was imperative to determine some harmful bacteria associated with smoked fish. Such an investigation was intended, among other things, to help establish if the level of microbial loads exceeded the permissible levels of the Ghana Standards Authority (GSA) and International Commission on Microbiological Specifications for Foods (ICMSF) of 1.0×10^7 CFU/g. The investigation was also intended to explore whether the activities along the fish smoking chain have effects on the bacteria quality of smoked fish. Such a study may also be useful in developing public health promotion campaigns aimed at creating awareness of bacteriological and smoked fish-based food safety. Outcomes from this study would also provide strategies to improve or minimise the bacteriological overload of smoked fish and also improve fish smoking activities along the processing chain.

2. MATERIALS AND METHODS

2.1 Study Design

The study was a comparative cross-sectional designed to explore 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 existed 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 in the Ashanti region of Ghana.

2.4 Sample Size and Sampling Procedure

Three fish processing sites (Yeji Kou, Yeji Konkoma and Yeji Nsuoano) were selected using the purposive sampling technique. 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:

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

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

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 were analysed using Microsoft Excel version 2016 (Microsoft, USA), Software Package for Social Sciences (SPSS) version 23 (IBM, USA), and an Interactive chi-square calculator (Preacher, K. J. 2001). The test significance chosen was 5%.

3. RESULTS

3.1 Fishery Sector of Yeji-Pru East District Based on Secondary Data Obtained from the Ministry of Finance and Economic Planning (MOFEP-Pru)

About 94% of all freshwater fisheries occur in developing countries, where they provide food and livelihood for millions of people. It also contributes to the overall economic well-being through export commodities, trade, tourism, and recreation [26]. Yeji is popularly known for the production of fish. This is based on the fact that Yeji is located just at the edge of Volta Lake. The Volta Lake masses about 140 fish species and is estimated to produce about 16 % of the total domestic catch and 85% of inland fisheries output [27]. The Yeji fishery sector provides jobs for about 46.3% of the people in the district either directly or indirectly in the areas of fishermen, fishmongers and traders [27]. The sector has therefore organized a series of training for fish mongers on processing methods intending to increase their income. It has also trained fresh fish traders on how to export fresh fish to big markets like Kumasi and Accra. The major challenge, however, is the depletion of the fish stock in Volta Lake as a result of overfishing and the use of unapproved fishing gear and nets for fishing.

In the study, secondary data which was obtained from the Ministry of Finance and Economic Planning (MOFEP-Pru), showed that the dominant species caught from 2009 to the mid-year of 2014 was catfish and tilapia with an overall total catch of 5,813,143. Table 1 and Fig. 1 show the yearly harvest of catfish (*Chrysichthys*) and tilapia (*Oreochromis*) from 2009 to the mid-year of 2014. The chi-square test of independence for the catfish and the tilapia groups showed that the distribution of their yearly harvest from 2009 to 2014 (mid-year) differed significantly (χ^2 =246842.539, DF = 5, *p*-value < 0.05). Of the 5,813,143 harvested fishes, a higher proportion of 65.9% (n=3,831,837) was catfish whereas tilapia constituted 33.8% (n=1,967,563) as presented in Fig. 1. This observation at the Yeji area in which the catfish was observed to be the dominant fish species among the fish landings is not consistent with the report on the situation at Lake Volta on the whole. For example, the Food and Agriculture Organization (FAO) has reported tilapia to be the dominant fish species (38.1%) among the fish landings at the Volta Lake, with the catfish species *Chrysichthys* being the second major fish species constituting 34.4% [28].

3.2 Processed Fish Production in Yeji-Pru East District Based on Secondary Data Collected from the Ministry of Food and Agriculture (MOFA)

In the study, secondary data which was obtained from the Ministry of Food and Agriculture (MOFA-Pru), showed that the overall processed fish (smoked and salted dried), captured as fresh fish weight equivalent that passed through the Yeji by the 3rd quarter of 2010, was 2,529,381 kg.

Table 1. Fish Production in Pru District from 2009 to 2014 (Mid-year)

		Frequency		Percentage	
Year	**Total**	**Chrysicthys**	**Tilapia**	**Chrysicthys**	**Tilapia**
2009	13743	7299	6444	53.1	46.9
2010	1973321	1178294	795027	59.7	40.3
2011	798781	588668	210113	73.7	26.3
2012	1973321	1178294	795027	59.7	40.3
2013	763742	636045	127697	83.3	16.7
2014(Mid-year)	290235	250536	39699	86.3	13.7
Total catch	**5813143**	**3831837**	**1967563**	**65.9**	**33.8**

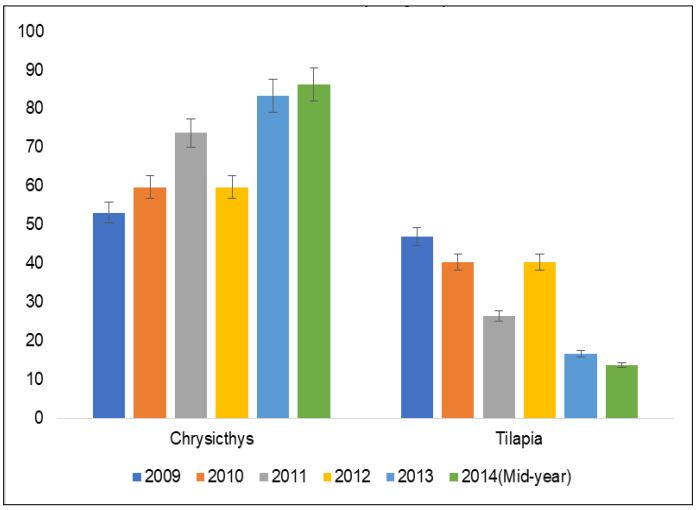

Fig. 1. Distribution of fish production in Pru district from 2009 to 2014 (Mid-year)

Table 2. Accumulated total landings in kilogrammes of catfish and tilapia (the fresh fish equivalent of processed fish) at the Yeji monthly in 2010

		Frequency		Percentage	
	Total	**Chrysichthys**	**Tilapia**	**Chrysichthys**	**Tilapia**
January	64009	36637	27372	57.24	42.76
February	1154688	679529	475159	58.85	41.15
March	100387	49879	50508	49.69	50.31
April	44119	22626	21493	51.28	48.72
May	80540	52190	28350	64.80	35.20
June	86834	52678	34156	60.67	39.33
July	70106	38499	31607	54.92	45.08
August	84712	52695	32017	62.20	37.80
September	84824	58683	26141	69.18	30.82
Total	**1770219**	**1043416**	**726803**	**58.94**	**41.06**

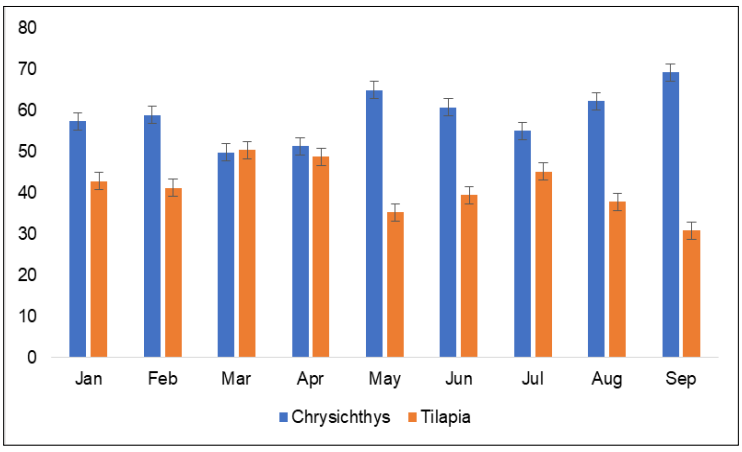

Fig. 2. Distribution of accumulated monthly total landings in kilograms of catfish and tilapia (fresh fish equivalent of processed fish) at the Yeji monthly in 2010

Table 2 and Fig. 2 show the monthly total number of processed (smoked and salted dried) catfish (*Chrysichthys*) and tilapia (*Oreochromis*), which was captured as fresh fish weight equivalent that passed through the Yeji market by the 3rd quarter of 2010. The chi-square test of

independence for the catfish and the tilapia groups showed that the distribution of their accumulated monthly total in kilogrammes (as the fresh fish equivalent of processed fish) at the Yeji weekly market in 2010 differed significantly (χ^2 = 10469.826, d.f. = 8, p< 0.05). Of the 1,770,219 kg total landings (the fresh fish equivalent of processed fish) at Yeji, a higher proportion of 58.9% (n=1,043,416) was catfish (χ^2 = 56627.904, DF = 8, p< 0.05) as presented in Fig. 2.

3.3 Socio-cultural Study of Fish Mongers

Table 3 presents the socio-cultural study of the selected fish mongers. The result revealed that all the fishmongers were between the age of 25 to 54 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 transported fish from landing sites using tricycles ("*Okada*"). 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 minutes (95% CI: 10.54 - 17.36). 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 3. 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

3.4 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 *E. coli* and *Staphylococcus* species on 89.6% (n= 43) of smoked fish species as shown in Fig. 3. 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 *E. 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 4.

3.5 Abundance of *Escherichia coli*

E. coli composition differed according to fish species as indicated in Tables 5 and 6. Pacific salmon (*Oncorhynchus sp*.) had an *E. 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, *E. 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, *E. coli* on catfish (*Chrysichthys auratus*) sampled from the studied areas were 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, *E. 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 ($p > 0.05$) as shown in Table 6.

3.6 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 7 and 8*. 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 ($p > 0.05$) as shown in Table 8.

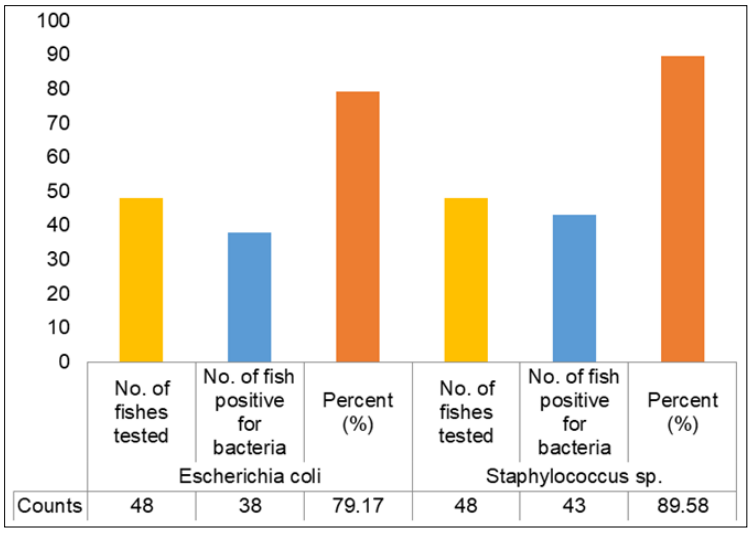

Fig. 3. Bacteria count for smoked fish

Table 4. 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

Table 5. 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 6. Interactive Chi-square Test for Goodness-of-fit on the average number of *Escherichia coli* colonies formed

Smoked fish species	Observed cfu/ml	Expected cfu/ml	χ2	DF	*p*
Salmon	99	87.33	2.618	2	0.27
	78				
	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				

Table 7. Colony forming units of *Staphylococcus spp.*

Sample	Site	Load (*cfu/ml*)			
Salmon	Yeji Kou	4.1×103	1.5×104	5.0×104	0.0×100
	Yeji Konkoma	3.6×103	1.1×104	2.8×104	0.0×100
	Yeji Nsuoano	4.4×103	1.5×104	6.0×104	0.0×100
Herrings	Yeji Kou	1.4×104	2.4×104	1.2×105	0.0×100
	Yeji Konkoma	1.4×104	2.5×104	1.4×105	2.5×105
	Yeji Nsuoano	1.4×104	2.2×104	8.0×104	0.0×100
Catfish	Yeji Kou	6.0×103	6.1×104	2.0×105	6.3×105
	Yeji Konkoma	5.6×103	3.4×104	1.2×105	1.8×105
	Yeji Nsuoano	6.2×103	4.1×104	2.1×105	7.0×105
Tilapia	Yeji Kou	5.2×103	2.4×104	1.5×105	2.0×105
	Yeji Konkoma	5.0×103	1.6×104	9.0×104	2.3×105
	Yeji Nsuoano	5.4×103	3.0×104	1.6×105	3.3×105

Table 8. 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	58.66667	2.057	2	>0.358
	50				
	65				
Total	176				
Herring	179	179	0.279	2	>0.867
	184				
	174				
Total	537				
Catfish	147	127.6667	7.462	2	<0.024
	104				

Smoked fish species	Observed cfu/ml	Expected cfu/ml	χ2	DF	*p*
	132				
Total	383				
Tilapia	92	91	3.451	2	>0.178
	78				
	103				
Total	273				

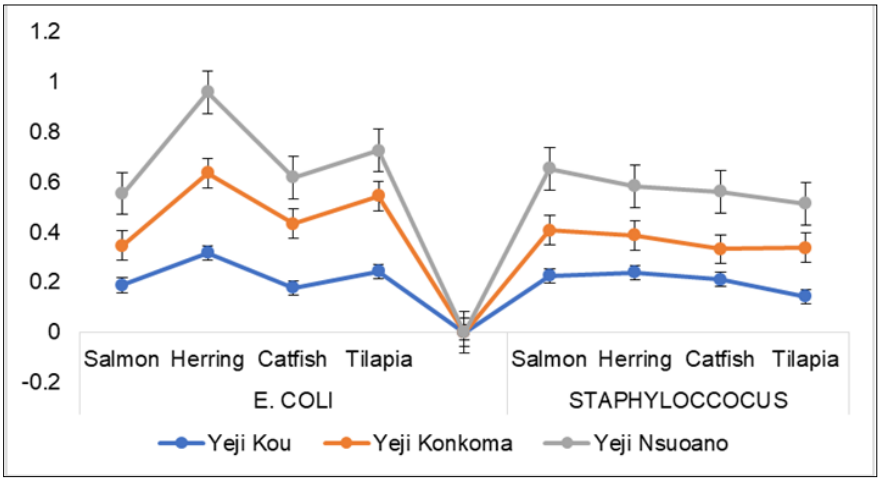

Fig. 4. Presents the microbial abundance of the sampled smoked fish across the three processing sites

Table 9. Comparing the *E.Coli* Abundance with GSA/ICMSF 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 10. Comparing the *Staphylococcus spp.* Abundance with GSA/ICMSF 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

3.7 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)

Fig. 4 presents the microbial abundance of the sampled smoked fish across the three processing sites. 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 and International Commission on Microbiological Specifications for Foods of 1.0 x 10^7 cfu/g as presented in Tables 9 and 10.

4. 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 fish to be smoked was 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*, *E. 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 [29,30]. This was in agreement with a study conducted by [31] 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 [32]. 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 [33], 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 [34,35].

Contamination of fish species by *E. coli* mostly results from the unhygienic condition of the smoking environment and materials used along the fish processing chain at large [36,37]. 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 *E. coli* [38].

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 [39], fish from the lake may be contaminated. The study by [39] on the quality of Volta Lake also demonstrated that the lake is significantly contaminated with microbes. This possibly contributed to the cause of the *E. coli* contamination of the smoked fish species as observed in the current study. The observation from this current study was also in agreement with earlier studies that revealed *E. coli* in smoked fish species processed in Ghana [22,33,40,41].

Staphylococcus species are commonly found in the nose, mouth and on skin of humans and animals. Contamination of foods by *Staphylococcus* species often results from poor handling. People who carry *Staphylococcus* species can contaminate food by merely touching it [42]. It was previously reported that the toxins produced by Staphylococcal contaminants are also resistant to heat, this is an indication that simple heat may not destroy these microbial contaminants [43,44]. 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 [44,45]. This finding was in line with other studies which detected *Staphylococcus* species on smoked fish from various markets in Ghana [22,33,40,41].

5. CONCLUSION

Although *Salmonella* and *Shigella* species were not detected in the fish species analysed in the current study, the high *Escherichia coli* and *Staphylococcus* species count sought to suggest that the smoked fish studied may be unfit for human consumption due to contamination by bacteria. However, a comparison with the Ghana Standards Authority and the International Commission on Microbiological Specifications for Foods showed that the bacteria load observed were lower than the permissible level for human consumption. Be that as it may, there is a need for effective foodborne infection or disease control measures to be planned and implemented to help improve the microbiological quality of smoked fish produced in the study area.

6. STUDY'S LIMITATION

The study's potential shortcoming was a linguistic barrier. An English-language questionnaire was used to collect data on sociodemographic characteristics. The questions were translated into Asante Twi for the participants who did not speak English. The interview process for those who could not speak any of the aforementioned languages was conducted through a dependable interpreter in the language of their choosing. When there was no trustworthy interpreter available in the language of her choosing, that particular fishmonger was not included in the study. Based on that, the authors suggested that a replication of the study could be done where more fishmongers could be incorporated in order to acquire more smoked fish samples.

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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