Microbial quality of fish along with the Tilapia, African catfish and Sardinella artisanal value chains in Kpong and James Town, Ghana

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Abstract. Aboagye E, Tano-Debrah K, Kunadu APH. 2020. Microbial quality of fish along with the Tilapia, African catfish and Sardinella artisanal value chains in Kpong and James Town, Ghana. Bonorowo Wetlands 10: 1–17. Fish from artisanal sources constitute the most critical animal protein in the Ghanaian diet. However, the availability and safety of fish on the Ghanaian market are unpredictable owing to potential rapid microbial growth, which results from high ambient temperatures and poor handling along the artisanal value chains. Little is known about the small-scale fish value chains and the key stakeholders’ food safety knowledge and processing practices. This study aimed at mapping out the artisanal fish value chains of Tilapia (Oreochromis niloticus), African catfish (Clarias gariepinus), and sardinellas (Sardinella aurita), and assessing the food safety knowledge and handling practices of key stakeholders along the selected value chains. A survey using semi-structured questionnaires involving 93 fishers, 40 retailers, 40 processors, and 120 consumers was carried out to investigate stakeholders’ knowledge and practices of food safety along the value chain. Samples of the selected fish species were taken along their respective value chains to test for the presence of safety indicators (Salmonella, Vibrio, and Listeria species), hygiene indicators (Staphylococcus aureus and Escherichia coli), and spoilage organisms (Pseudomonas spp. and Proteus spp.). The mean scores for food safety of retailers, processors, and consumers were generally insufficient at 55%, 43%, and 67.3%, respectively. The stakeholders also scored poorly in their handling practices, with mean scores of 41.2%, 63.0%, and 58.6% for fishers, processors, and consumers, respectively. Estimated fish losses were highest at the retailer and consumer stages of the value chain, with reported injuries as high as 35 to 100%—pathogens such as Clostridium perfringens, enteropathogenic Escherichia coli, Staphylococcus aureus, Listeria spp. and Aeromonas sobria were isolated from fresh and processed ready-to-eat fish samples. Salmonella spp. and Vibrio spp. were not detected on any samples tested. Mesophilic counts ranged from 7.96 ± 0.68 to 2.95 ± 0.23 log cfu/g reported from fresh fish samples, with similarly high fecal coliform counts averaging 3.11 log cfu/g. Processed fish samples had average total counts, fecal coliform counts, and yeasts and mold counts of 3.11, 2.27, and 2.45 log cfu/g, respectively. Proteus vulgaris and Proteus mirabilis were the predominant spoilage organisms present in almost all the fresh fish samples. This study provided much-needed insight into the unsatisfactory safety and quality of artisanal fish on the Ghanaian market and the specific microorganisms associated with them along the value chain. It also established the link between the food safety knowledge and handling practices of stakeholders within the value chain and the actual quality and safety of fish on the market.

Keywords: Microbial quality, fish, tilapia, African catfish, sardinella

INTRODUCTION

Fish contributes about 40–60% of the animal protein supply in the Ghanaian diet and is recognized as the most important source of animal protein in every part of the country (MoFAD 2011). The cost of fish constitutes 22.4% of the food expenditure in all Ghanaian households and 25.7% in low-income families (BOG 2008). Fishing communities, often some of the poorest in the country, depend heavily on fish and related activities for their livelihoods (FAO 2013). As an agricultural commodity, fish is essential in ensuring food security, especially among the poorest in the country.

However, the importance of fish as a food security commodity in Ghana is affected by high post-harvest losses. In the high tropical temperatures of Ghana, fresh fish spoilage can be remarkably rapid after capture. Fish perishability is aggravated by its intrinsic properties, such as high water activity, near-neutral pH, and high digestible protein content, all of which provide conducive conditions for microbial proliferation (Ghaly 2010). Microbial activity alone accounts for the spoilage of 30% of landed fish worldwide (Bataringaya 2007). In Ghana, 10-30% of the artisanal catch sold for less than its actual worth due to quality deterioration (Akande and Diei-Ouadi 2010).

In terms of safety, fish has been implicated in several outbreaks of food-borne infections. It is a potential vehicle for food-borne diseases such as cholera, listeriosis, salmonellosis, and others (Popovic et al., 2010; Costa 2013; Akoachere et al. 2009). Many spoilage microorganisms, known to be opportunistic pathogens, including Pseudomonas spp. and Proteus spp., have also been associated with fish (Ikutegbe and Sikoki 2014; Popovic et al. 2010; Tryfinopoulou et al. 2007; Viji et al. 2014).

Therefore, poor fish quality has dire consequences, which transcend the loss of an important protein source. Substantial economic injuries are incurred annually because of losses in production volume, monies spent in treating foodborne infections, and human resources lost during the illness and the incidence of death (Akande and Diei-Ouadi 2010). With an already existing annual deficit of
320,000 Mt in Ghana’s fish requirements, fish quality loss is a problem that requires urgent attention (BOG 2008).

The Artisanal fishery in Ghana contributes 70% to 80% of the total marine fish production and is the principal supplier of fish on the local market (Amador et al. 2006: FAO 2013). There is, however, scanty literature on the structure of the artisanal marine and freshwater fish value chains. The few reported studies do not clearly depict the key players in these value chains and how their knowledge and practices concerning food safety impact the final quality of the fish that reaches the consumer. The occurrence of key pathogens associated with fish and how they are affected by processing and handling along the value chain is also sparsely documented. Again, the specific spoilage organisms associated with fish sourced from Ghanaian waters and their occurrence along the value chain are unknown. These gaps in knowledge are of great concern given the severity of the consequences associated with fish spoilage, especially regarding food security.

Also of concern is the poor and unsanitary conditions prevailing in most fish landing sites in Ghana. It becomes even more necessary for key actors or stakeholders within the fish value chain to be aware of and consistently implement proper handling and storage of fish before processing and distribution to consumers. Therefore, it is vital to investigate the association between the food safety knowledge and practices of artisanal fish stakeholders and the actual microbiological quality of their fish. This would provide evidence and insight into how these stakeholders, through their cultural and food safety-related practices, impact the extent of fish losses and the microbiological quality of artisanal fish on the local market.

The objectives of this research were: (i) To map out and document the artisanal value chains of Tilapia (Oreochromis niloticus), African catfish (Clarias gariepinus), and sardinellas (Sardinella aurita) in Kpong and James Town. (ii) To test fish samples along the artisanal fish value chain for the presence of safety indicators (Salmonella spp., Vibrio spp. and Listeria spp.), hygiene indicators (Staphylococcus aureus and Escherichia coli), and spoilage organisms (Pseudomonas spp. and Proteus spp.). (iii) To establish the link between food safety knowledge and practices of stakeholders in the artisanal fish value chain and the microbiological quality and safety of fish. (iv) To determine the potential implications of poor fish microbiology on food security availability and safety components.

MATERIALS AND METHODS

Study design

The study was in two parts. The first part was an initial cross-sectional survey that traced the local artisanal fish value chain. It also assessed the food safety knowledge and practices of key players or stakeholders within the identified value chain through questionnaire surveys. Participation in the study was voluntary, and anonymity and confidentiality of the response were ensured. Ethical clearance was obtained from the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR- IRB), University of Ghana.

The second phase was microbial analyses in the laboratory. The study examined the microbiological quality of three locally consumed fishes at different stages of their respective value chains. The Sardinella was used as a case study for marine fishes, while Tilapia and the African catfish were used for freshwater fishes. These fishes were selected based on their availability and popularity among different socio-economic groups within Ghana.

Sampling and data analysis for a cross-sectional survey

The fishermen were the first point of contact fishers the individual fish value chains. They asked who supplied resources necessary for their expeditions and which groups of people they handed their catch to. The trail was then followed to identify the next group of stakeholders until the fish reached the final consumer. All the different kinds of processing the fish subjected to and the major fish markets, where fish were either retailing or wholesaled, were also identified in the process. Consumers were recruited because they had purchased raw, unprocessed fish from local informal markets at any time within the past six months.

Tilapia and the African catfish were traced from stakeholders in Kpong, Senchi, and Ayikpala, all of which were artisanal fishing communities located around the Volta Lake in the Eastern region of Ghana. Stakeholders in the Sardinella value chain were interviewed along with James Town and Choker’s coastlines in Accra and the major fish markets, Salaga, Madina market, and Kaneshe in Accra and Tuesday market in Mamprobi, a suburb of Accra (Figure 1).

A total of 293 stakeholders in the value chain were interviewed with four semi-structured questionnaires. These included 93 fishers, 40 retailers, 40 processors, and 120 consumers. The interviewer entered all questionnaires were administered in the local language, and gave responses. All questionnaires were pre-tested before use. The questionnaire designed for fishers evaluated food safety practices based on four questions, while retailers/wholesalers, processors, and consumers were assessed based on eight items on the survey. Knowledge about food safety was also assessed based on seven items on the wholesaler, retailer, processor, and consumer survey.

In both the knowledge and practice sections of the surveys, categorical responses (yes/no/don’t know) and open and more detailed responses were used. Each correct answer within the categorical responses carried a score of 1, while correct responses in the open-ended questionnaires carried a score of 2. ‘Wrong’ or ‘don’t know answers’ were given a score of zero. For each respondent, the questions’ score was summed up and converted into percentages (0 to 100). A representative score of 70% and above was considered “sufficient knowledge/practice,” while a score of <70% was considered “insufficient knowledge/practice.” The scoring system applied here was adapted from similar studies by Osaili et al. (2013) and Zanin et al. (2015).
Figure 1. A map of towns and markets from which samples were obtained

Table 1. Study design for microbiological analyses

| Stakeholders | Product  | Fish type (N) | Tilapia | African Sardinella |
|--------------|----------|---------------|---------|--------------------|
| Fishers      | Freshly landed fish | 1x2 | 1x2 | 1x2 |
| Wholesalers  | Fresh fish | 1x2 | 1x2 | 1x2 |
| Retailers    | Fresh fish | 1x2 | N/A | 1x2 |
| Processors   | Raw fish | 1x2 | 1x2 | N/A |
| (fermented/salted) | Raw fish | 1x2 | N/A | N/A |
| Processors (Salted/dried) | Raw fish | 1x2 | N/A | N/A |
| Processors (Grilled) | Raw fish | 1x2 | N/A | N/A |
| Processors (Smoked) | Raw fish | 1x2 | 1x2 | 1x2 |
| Processors (Smoked/dried) | Fermented/dried | 1x2 | 1x2 | N/A |
| Processors (Salted/dried) | Salted/dried | 1x2 | N/A | N/A |
| Processors (Smoked) | Smoked | 1x2 | 1x2 | N/A |
| Processors (Smoked/dried) | Fried | 1x2 | 1x2 | N/A |
| Processors (Salted) | Salted | 1x2 | N/A | N/A |
| Processors (Smoked/dried) | Sun-dried | N/A | N/A | 1x2 |

Note: N/A: Not applicable (samples were not typically found at that stage of the value chain)

Sampling for microbiological analyses

Sampling was done at different value chain stages for each type of fish. The samples were collected on at least two separate occasions and from different individuals provided they sourced their fish from areas in and around Kpong in the case of Tilapia and catfish, and James Town in the case of the sardinellas.

Samples were collected into sterile stomacher bags, appropriately labeled, and transported in thermos ice chests disinfected with 70% alcohol. All fresh and raw samples were transported on ice and analyzed in the laboratory within 4 hours of sampling. Processed fish samples were analyzed within 24 hours of collection. To prevent cross-contamination, processed fish were not sampled on the same day as the raw and fresh fishes.

Sampling of fish from Fishers

Sampling was done by convenience; sampling of the landed fish was done by asking the fishers to select the fish of interest from their catch randomly. Sampled fish was then transferred into a sterile stomacher bag handled by the gloved hands of the researcher. The bag was sealed and put into the thermos ice chest.
Sampling of fish from wholesalers and retailers

Fresh fish samples were purchased from wholesalers at the fish landing site and retailers at the informal fish markets in Salaga and Madina. The fishes obtained from individuals far removed each other in the market. The wholesaler or retailer was asked to select the fishes in the same manner randomly she would handle them when selling to a consumer or customer.

Sampling of fish from Processors:

Sampling here done at the processing site. Where it was available, samples of the raw fish intended for processing were also collected, and their storage temperature was measured with a thermocouple (Thermo scientific). However, such samples were collected and transported in a container separate from the processed fish samples. Processed fish in storage or ready to be served to consumers were randomly selected by the processor and sealed in sterile stomacher bags for transport on ice to the laboratory. The samples were stored in a cold room at 4°C for no longer than 24 hours when they could work on immediately.

Microbiological analyses

Samples were analyzed for the total count or concentration of aerobic mesophiles, coliforms, staphylococci, yeasts and molds, and Clostridium perfringens. The fish samples were also analyzed for the presence of Escherichia coli, Salmonella spp., Vibrio cholerae, Vibrio parahaemolyticus, Staphylococcus aureus, Pseudomonas spp., and Listeria spp.

Sample preparation

Ten grams of each sample was aseptically weighed into sterile stomacher bags with the addition of 90 mLs peptone water. Because of their smaller sizes, juvenile sardinelunas are considered the whole. In the case of tilapia and catfish, bits of the surface tissues, gills, gut, and muscle from the loin (thickest part of the fish muscle) were taken with sterile scissors and aseptically weighed to obtain the final mass. The weighed samples were then homogenized for up to 2 minutes in a Seward stomacher blender. Serial dilutions for the various microbial counts earlier mentioned were then carried out according to methods described by the International Commission for Microbiological Specifications for Foods (ICMSF, 1985).

Aerobic mesophilic count

In a method described by the ICMSF (1985), homogenates of fish samples were serially diluted, and 0.1 mL of three dilutions were pipetted into sterile disposable Petri-dishes. Twenty-milliliter portions of Plate Count Agar (PCA) (Biolab-Merck) were then poured on the inoculum using the pour plate technique. The set PCA dishes were incubated inverted at 20-25°C for 48±2 hours to account for psychrophiles present. Two replicates of at least two dilutions with 25-250 discrete colonies were enumerated following incubation. All counts were reported as the logarithm to base 10 colony-forming units per gram (log cfu/g).

Enumeration of total staphylococci and detection of S. aureus

Three serial decimal dilutions of the samples were plated using the pour plate technique on Baird-Parker agar (Oxoid CM275) supplemented with egg yolk tellurite emulsion (Oxoid). After incubation at 35-37°C for up to 48 hours, plates containing 20-200 typical staphylococcal colonies (black, circular) were counted. Up to 5 colonies on each plate subcultured on nutrient agar (Oxoid CM003), gram stain, and tested for catalase activity. All typical staphylococci were catalase-positive and were gram-positive stacked cocci. Staphylococcus aureus colonies were circular, convex, grey-black to jet-black with an off-white margin. They were also gram-positive cocci, catalase-positive, and coagulase-positive (APHA 2001).

Enumeration and detection of faecal coliforms and E. coli

Three serial decimal dilutions of the fish-peptone water homogenate were poured plated on Levine Eosin Methylen Blue (EMB) agar (Oxoid CM0069) and incubated at 35°C for 24 hours. Plates with 20-200 Purple colonies were counted and subcultured on MacConkey agar (Oxoid). Faecal coliforms fermented the lactose (pale pink color on MacConkey) and were gram-negative rods.

Escherichia coli colonies appeared circular, dry, and flat with a metallic sheen. Five of such provinces subcultured on Sorbitol MacConkey (SMAC) agar (Merck), where they appeared as pinpoint, pale pink colonies. These suspect colonies were then purified on nutrient agar and transferred to Triple sugar iron agar (TSI) slants, Simon’s citrate slants, and Sulphur-Indole-Motility (SIM) agar. E. coli colonies were indole positive, gas positive, H2S negative, citrate negative, and fermented glucose and sucrose.

All presumptively identified E. coli colonies were confirmed with API 20E and serotyped using a serotyping kit. Klebsiella spp. often mimicked E. coli on EM plates but had a moist appearance, with or without a metallic sheen, and was also slimy when touched with an inoculating loop. Suspect colonies were purified on nutrient agar and confirmed with API 20E (APHA 2001).

Clostridium perfringens count

Spread plates of serial dilutions were made using 0.1 mL aliquots on TSC (tryptose-sulfite- cysteine) agar (Oxoid CM0587) supplemented with egg-yolk and TSC supplement (Oxoid). After the agar had dried slightly, the surface was overlaid with 5mL of TSC agar and incubated upright in an anaerobic jar containing an aerobic gas generating kit (Oxoid anaerogen) and incubated at 35-37°C for 24 hours. Plates containing 20-200 black colonies with opaque halos were selected and counted. To confirm presumptive positive Clostridium perfringens colonies, 5 black colonies were selected and tested for motility in SIM agar and nitrate reduction in nitrate broth (Fluka 72548). Clostridium perfringens reduced nitrate and was nonmotile (APHA 2001).

Detection and enumeration of yeasts and moulds

The homogenate’s serial decimal dilutions were pour plated in Malt Extract Agar (MEA)- Oxoid CM0059,
supplemented with 10% lactic acid and incubated at 25°C for up to 5 days. Plates containing 20-200 colonies were counted.

Detection of Pseudomonas species:
In a method described by Tryfinopoulou et al. (2001), dilutions of the homogenate were poured plated in Pseudomonas agar (Oxoid CM0559) with 5ml of glycerol and a vial of pseudomonas CFC (Cephaloridine-fucidine-ecetrimide supplement (Oxoid). They incubated at 25°C for 24 hours and 48 hours. Pseudomonas aeruginosa appeared as straw-colored colonies with green pigmentation. Presumptive positive colonies were subcultured on nutrient agar and tested for oxidase activity. Pseudomonas spp. were Gram-stained (gram-negative rods) and confirmed with API 20E.

Detection of Vibrio species
Twenty-five grams of fish samples were weighed and homogenized in 225ml of alkaline peptone water. The homogenate was aseptically dispensed as 10ml aliquots in lightly capped test tubes and incubated for 18-24 hours at 35±C. The pre-enriched samples were vortexed, after which a 3 mm loop (about 0.1ml) was aseptically taken and streaked unto well-dried Oxoid CM0333 Thiosulphate Citrate and Bile salts Sucrose (TCBS) agar plates. The plates were incubated (inverted) at 35-37°C for 18 to 24 hours or until satisfactory growth.

Suspect Vibrio cholerae colonies on TCBS agar were large, smooth, flat, and yellow, while Vibrio parahaemolyticus were smaller, green, and round. Aeromonas spp. could mimic both appearances on the TCBS agar. All suspect colonies were purified on nutrient agar and tested for oxidase activity. Colonies found to be oxidase positive were purified further on nutrient agar and confirmed with API 20E (APHA 2001).

Detection of Salmonella species
Twenty-five grams of fish samples were homogenized in 225ml of selenite broth, dispensed into loosely capped sterile test tubes as 10ml aliquots, and incubated for 18-24 hours at 35°C to recover injured cells. Three drops of the pre-enriched culture were evenly inoculated on plates of Modified Semi-solid Rappaport Vassiliadis medium (MSRV, Oxoid CM1112). A loop full of the pre-enriched culture was also streaked on dried plates of Salmonella-Shigella agar (SSA, Park scientific M0240). Growth on both media was examined after 18-24 hours at an incubation temperature of 35°C.

Presumptive positive Salmonella colonies appeared on SSA as straw-colored with or without black centers. Proteus spp. often swarmed the SSA plates, appeared as black colonies, and had a very foul smell. Suspect Salmonella colonies were isolated and purified on nutrient agar for biochemical testing.

Suspect Salmonella growing on the MSRV were greyish and appeared motile. A growth sample was streaked onto a dried SSA plate with a sterile loop and incubated overnight. Salmonella-like colonies observed were streaked unto Nutrient agar for purification. All purified colonies were tested for urease reactions on Urea agar slants and TSI agar.

Gram-negative rods with adverse urea reactions were confirmed using the API 20E kit (APHA 2001). All identified Proteus species were tested for indole reaction to differentiate P. mirabilis or P. penneri from P. vulgaris.

Detection of Listeria species Twenty-five-gram fish samples homogenized in 225ml of Merck Listeria Enrichment Broth (LEB) were distributed into loosely capped test tubes as 10 ml aliquots. After 24 hours of incubation at 35°C, 1ml of the LEB-fish homogenate was septically transferred into 9ml of Fraser broth (Oxoid CM0895). This was incubated at 35±2°C for another 24 hours. A loop full of the enriched homogenate was then streaked unto well-dried plates of Chromogenic Listeria agar (LCA, Oxoid CM1017), incubated at 35±2°C for 24 hours or until satisfactory growth was satisfactory. Suspect Listeria colonies appeared as blue-green colonies surrounded by an opaque halo.

Selected colonies on the LCA plates were purified on 5% sheep blood agar (Oxoid). Presumptive Listeria colonies appeared whitish on blood agar and displayed β-hemolytic activity. Discrete colonies from the blood agar were also tested for motility on SIM agar, catalase activity, Gram’s reaction. Presumptive positive Listeria were catalase-positive, gram-positive short rods and displayed umbrella motility in SIM agar (APHA 2001).

Physical and chemical analysis of intrinsic properties of fish
Temperature
The fish (both fresh and processed) temperature was measured with a thermocouple (Hanna Instruments) calibrated in hot water at 100°C and ice water at 0°C on each sampling day. The thermocouple probe was first disinfected with 70% ethanol before measuring the temperature at the midsection and tail regions. The average of the three readings was recorded and reported as mean± Standard deviation.

pH
The moisture content of the fish sample was first determined using the standard method described in I.S 14950: 2001 fish dry and dry salted. If the fish was, for example, found to contain 20% moisture, it implied that every 10g of the sample weighed had 8g of dry matter. To obtain 10g of dry fish matter, 12.5g of the sample was considered and homogenized in 87.5g of deionized water. The pH of the homogenate was then measured with a glass electrode pH meter. The average of three readings was recorded and corrected for temperature differences (I.S 14950: 2001).

Determination of risk factors along the fish value chain
To establish the link between the food safety knowledge and practices of stakeholders and the actual quality and safety of the fish, a flow chart was designed based on evidence from the survey and the laboratory microbiological analyses.

The risk of poor handling is determined from the food safety knowledge scores and handling practices determined
from the survey. Also, the risk of fish spoilage and pathogenic contamination was established by the presence of spoilage organisms and pathogens, respectively, from fish sampled at the various stages of the value chain. Finally, temperature abuse and poor hygiene were determined by recorded fish temperatures and counts of hygiene indicator microbes, respectively.

**Statistical analysis**

Using their demographic characteristics as covariates, a binary logistic regression was used to determine the possible predictors of stakeholders' food safety knowledge and handling practices. Descriptive statistics such as means, standard deviations, and frequencies were used to analyze microbial counts. The means were data from three independent experiments for fresh and processed fish microbial counts. Analyses of variance, ANOVA (one-way) were used to assess the significance of differences between counts of microbes obtained from different stakeholders and between the counts sourced from the different value chains (marine and freshwater).

The percentage prevalence of isolated microorganisms was determined using Cross tabulations. Pearson’s chi-square was used to test the association between counts of microbes obtained from different stakeholders and the estimated fish losses. All statistical analyses were done using IBM SPSS version 21, Minitab version 14, and Microsoft Excel (2010).

**Assumptions and limitations**

Fishers were generally harder to track and interview because they often landed at different times of the day and were constantly busy negotiating, sorting, or selling their catch at the time of the interviews. To reduce the time required in interviewing them, fishers were not assessed on their food safety knowledge. Instead, it was assumed that their practices were more important, given that they typically handed over their catch immediately after landing. The study also assumed that the reported practices of the stakeholders were their actual practices.

**RESULTS AND DISCUSSION**

**Demographic characteristics of stakeholders in the Kpong and James Town artisanal fish value chain**

Every stage in the artisanal fish value chain was dominated by women, except the fishers, who were all male. Wholesalers and fresh and processed fish retailers were predominantly female (87.5%). Processors were also mostly female, recording 97.5% in all areas surveyed in this study. The demographic characteristics of the stakeholders are depicted in Table 2.

It was interesting to note that most stakeholders were above 40 years of age, except consumers. This, as explained by the stakeholders, was primarily because a significant amount of capital and social connections were required to enter the fish business. As many as 32% of fishers had never received a formal education. Similarly, 17% of wholesalers and retailers, and 27% of processors, also had no formal training.

Fishers in James Town were predominantly ethnic “Ga” while those in Kpong mainly were of “Ewe” and “Ga-Dangbe” descent.

| Table 2. Demographic characteristics and profile of stakeholders within the fish value chain |
|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Biodata** | **% Stakeholder (N)** | **F'man (N=93)** | **Whol/Ret (N=40)** | **Pros (N=40)** | **Cons (N=120)** |
| **Sex** | | | | | |
| Male | 100 | 12.5 | 2.5 | 9.2 | 94.2 |
| Female | 0 | 87.5 | 97.5 | 90.8 | 15.8 |
| **Age** | | | | | |
| 18-24 yrs | 22.6 | 25 | 15 | 42.5 | 15.8 |
| 30-39 yrs | 26.9 | 35 | 30 | 32.5 | 15.8 |
| >40 yrs | 50.5 | 40 | 55 | 25 | 15.8 |
| **Ethnicity** | | | | | |
| Ada | 24.7 | 17.5 | 10 | 2.5 | 4.2 |
| Krobo | 0 | 0 | 15 | 4.2 | 5.8 |
| Ewe | 39.8 | 47.5 | 32.5 | 15.8 | 8.3 |
| Akan | 3.2 | 7 | 2.5 | 53.3 | 15.8 |
| Ga | 32.3 | 27.5 | 40 | 15.8 | 15.8 |
| Northerner | 0 | 0 | 0 | 8.3 | 5.8 |
| **Religion** | | | | | |
| Muslim | 74.2 | 92.5 | 92.5 | 94.2 | 94.2 |
| Traditional African | 10.8 | 2.5 | 5 | 2 | 0 |
| None | 12.9 | 0 | 2.5 | 0 | 0 |
| **Education** | | | | | |
| None | 32.3 | 17.5 | 27.5 | 19.1 | 19.1 |
| Pri/Midsch/SHS | 65.6 | 80 | 79.5 | 65.9 | 65.9 |
| Tertiary | 2.2 | 2.5 | 0 | 14.7 | 4.3 |
| **Longevity in fisheries and related activities** | | | | | |
| 6-10yrs | 11.8 | 12.5 | 12.5 | 5 | 15.0 |
| 11-13yrs | 11.8 | 12.5 | 17.5 | 7 | 15.0 |
| >16yrs | 20.4 | 15.0 | 17.5 | 7 | 15.0 |
| 6-10yrs | 47.3 | 35.0 | 37.5 | N/A | N/A |

Note: N/A: not applicable;  F'man: fishers; Whol/Ret: wholesaler/retailer; Pros: processor; Cons: consumer
Mapping out the artisanal fish value chains in Kpong and James Town

The value chains of tilapia and the African catfish, traced from the artisanal fishers in Kpong, are depicted in Figures 2-3, respectively. The value chain began with fishes, most of whom own their canoes and a few who rented the canoe daily or weekly. Tilapia and the African catfish (freshwater fishes) were often sold at landing by fishers to women who in turn sold the fish either on wholesale or retail to consumers, processors, and other small-scale retailers. Many of these small-scale retailers sold the fresh fish in vehicular traffic to travelers on the Kpong-Tema roads. At the same time, some also sold the fresh fish in distant markets like the Madina and the Makola markets in Accra and markets in Tema. Also, at retail, a secondary group of stakeholders was depicted in Figures 2-3 as “Fish cleaners.” This group of people, who included both men and women, would often hang around the landing site and render the service of gutting, scaling, and sizing of the tilapia and catfish bought by consumers. This is important because these individuals increased the number of handlers along the value chain and were a potential source of recontamination.

Tilapia was typically processed by smoking, salting (“Koobi”), grilling, frying, and fermenting (“Momone”). The African catfish was also mainly processed by smoking, frying, and fermenting. Fried tilapia and catfish were retailed directly by processors who hawked in traffic on the Kpong-Tema Highway. The Grilling of Tilapia was mainly carried out at night and retailed directly by the processors as street food. Smoked and salted tilapia often sold wholesale to retailers who sold the fish to consumers previously mentioned local markets. However, it was still possible to purchase salted and smoked tilapia directly from the processors. This also applied to smoked and fermented catfish. To keep smoked tilapia or catfish from rapid deterioration, processors and retailers reported that they re-smoked or reheated the fish daily until it sold to consumers. Nonetheless, most tilapia and catfish were reportedly bought by consumers in their raw or unprocessed state. Most consumers, however, reported buying sardinellas already processed. The absence of a frozen fish value chain, the Tilapia value chain, and the African catfish’s value chain was significant. Cold store operators in and around the study sites reported having obtained their fish from inland fish farms rather than artisanal fishers. If any of the artisanal catch, therefore, very little ended up in frozen storage or cold stores before reaching the consumer.

The value chain of the sardinella, which is sourced from marine habitat, was markedly more complicated than the freshwater fishes. This value chain is depicted in Figure 4. This value chain also began with the fishers but had a unique and exciting group of stakeholders, the fish queens known locally as “Lonye.” These women appeared to wield great influence throughout the sardinella value chain, and in fact, the value chains of most fishes landed at the James Town fishing harbor.
The Fish Queens were typically the captain’s wives of the fishing canoes, and in many cases, these women also owned the boats. They also reportedly pre-financed some fishing expeditions by providing fishing gears and pre-mix fuel for the outboard motors. When the fish queen held the canoe or pre-financed the operation, she handed the most significant percentage of the catch or the entire catch. The fish queens reportedly had arrangements with several fishermen on different canoes and were able to sell large quantities of fish pooled from different fishermen. A similar system has reported in the Lake Victoria fisheries bordering Uganda (FAO 2013). Many fishermen also owned their canoes or worked for other men who financed their expedition, much as the fish queen did. They, however, also sold their fish to the fish queens and scarcely engaged themselves in selling the fish directly to consumers or other stakeholders. The roles of these women have also been described by Akrofi (2002), who referred to them as “fish mummies.”

Sardinelas were typically hot smoked, sun-dried, and occasionally fried. Smoking was, however, the primary mode of processing. Smoked sardinelas were retailed on local markets like Madina, Kaneshi, and Makola, all in Accra and distant markets such as Kumasi in the middle belt of the country and the Northern regions as well.

Fish smokers, who often processed their fish in large quantities to make efficient fuel and labor, typically purchased their raw fish from the fish queens. With the order placed, the fish was carried in cane baskets on young boys’ heads to the processing site or by public transport if the site was too far off. The sardinelas were thus ready to be smoked within a few hours after landing. It should be noted that very little smoking was carried out on the beach of James Town. Most of the sardinelas landed in James Town were smoked on the beach of “Chorkor,” a fishing settlement about 50 minutes’ walk (4km) from James Town. Many of the fishermen in James Town were also reportedly resident in “Chorkor.” Chorkor was thus the primary hub of fish smoking; many wholesalers and retailers from different parts of the country were reported to purchase smoked fish in this town for resale in local and international markets.

When sufficient quantities could not be obtained from artisanal sources during lean seasons, fish smokers mainly reported buying frozen sardinelas from cold stores. Like tilapia and catfish, sardinelas in frozen storage are not obtained from artisanal sources. Cold store operators bought their sardinelas from industrial fishing trawlers at the Tema Port.

Contrary to the practice in most developed countries, wholesale and retail of fresh artisanal sardinel, tilapia or catfish was rarely measured out in costs per weight of fish. At the wholesale level, fish is sold per basket or crate. The baskets were filled at the discretion of the fishers or wholesalers and not weighed. Retail was done by counts or numbers in the case of tilapia and catfish, while sardinelas retailed in handfuls. This practice invariably led to frequent handling of the fish by both retailers and consumers, thus introducing opportunities for contamination. It also led to variations in prices between different retailers, resulting in longer bargaining times.

In the case of Tilapia and the African catfish, the price could vary considerably depending on the size of the fish in the crate. The fishes were therefore sorted by size and species before the sale. Stakeholders also used sorting in the marine fish value chain. However, smaller fish species in the marine fish value chains were often sold without sorting. This was particularly true for sardinelas mixed with Anchovies at the landing and the wholesale level, mainly because Anchovies were similar in size and could be utilized in place of sardinelas in most recipes. Processors, therefore, smoked these two species together but separated them after smoking. Once smoked, the sardinelas and anchovies were sorted out and sold separately to consumers and other retailers, mainly because sardinelas fetched a higher price. However, this sorting step after processing increases handling and could introduce post-processing contamination.
Food safety knowledge and practices among stakeholders

The mean knowledge score for all stakeholders was found to be $6.0\pm2.3$ (60%), while the mean practice score was $5.64\pm2.8$ (56%), generally suggesting insufficient levels of food safety knowledge and practice. The exception was with the retailers who had a mean practice score of $7.85\pm1.64$ (79%), which suggested sufficient levels of food safety practices.

Fishers, processors, and consumers had mean practice scores of $4.12\pm2.99$ (41%), $6.30\pm2.04$ (63%), and $5.86\pm2.42$ (59%), respectively. Mean knowledge scores of retailers, processors, and consumers were found to be $5.50\pm1.99$ (55%), $4.30\pm2.05$ (43%), and $6.73\pm2.02$ (67%), respectively. Table 3 displays the responses on the food safety knowledge of stakeholders along the value chains of the fishes used in this study.

Table 3 generally revealed good knowledge among all stakeholders regarding indications of fish spoilage, proper ways of handling fish and preventing fish spoilage. Table 4 also displays the questions which assessed fish handling practices and showed that the stakeholders reported practices that supported this knowledge.

However, it can be argued that this knowledge is fundamental to their trade and essential to prevent economic losses. It is traditional knowledge passed down through generations, as evidenced by the fact that 87% of processors and retailers reported that their skills are were acquired through family traditions. Therefore, it can be deduced that they had no genuine knowledge of the actual causes of fish spoilage.

Table 5 displays the actual responses given by the stakeholders when asked about the cause of fish spoilage.

### Table 3. Responses to questions on food safety knowledge

| Questions                                      | % Stakeholders who gave correct or wrong responses |
|------------------------------------------------|--------------------------------------------------|
| Are you able to identify spoiled fish?         | Ret/whole (40)  Processors (40)  Consumers (120) |
|                                                | Correct  Wrong  Correct  Wrong  Correct  Wrong  Correct  Wrong |
| Are you able to identify spoiled fish?         | 87.5   12.5  100.0   0.0   92.5   7.5 |
| What are the indications of fish spoilage?     | 69.7  30.3  82.5  17.5  75.8  24.2 |
| What causes fish spoilage?                    | 67.5  32.5  40.0  60.0  87.5  12.5 |
| Could handling contribute to spoilage?        | 77.5  22.5  87.5  12.5  83.3  16.7 |
| What are some of the bad handling practices    | 77.5  22.5  60.0  40.0  83.3  16.7 |
| Describe the condition of infected fishes      | 32.5  67.5  17.5  82.5  5.5  94.5 |
| Can eating a diseased fish cause illness       | 22.5  77.5  20   80   52   48 |
| What illnesses are caused by eating spoiled fish? | 2.5   97.5  20   80   49.7  51.3 |
| How is fish spoilage prevented?                | 95.0  5.0   77.5  22.5  93.4  6.6 |
| Average scores                                | 5.50±1.99  4.30±2.05  6.73±2.02 |

### Table 4. Responses to questions on food safety practices

| Questions                                      | % Stakeholders |
|------------------------------------------------|----------------|
| Do you inspect raw fish before purchasing/selling? | Fishers (93)  Whole/retail (40)  Processors (40)  Consumers (120) |
|                                                | Correct  Wrong  Correct  Wrong  Correct  Wrong  Correct  Wrong |
| Do you inspect raw fish before purchasing/selling? | N/A  N/A   85.0   15.0   80.0  20.0   81.0  18.2 |
| What indications of spoilage do you look out for? | N/A  N/A   85.0   15.0   75.0  25.0   75.8  24.2 |
| How do you transport raw fish?                 | 52.7  47.3  85.0  15.0  45.0  55.0  20.8  79.2 |
| How do you prevent spoilage?                  | 52.5  47.5  95.0  5.0  81.5  22.5  N/A  N/A |
| How do you store your fish?                   | 48.0  50.0  82.5  17.5  97.5  2.5   93.3  6.7 |
| What do you do with spoiled fish?              | N/A  N/A   5.0   95.0   32.5  67.5  90.8  9.2 |
| Average scores                                | 4.12±2.99  7.85±1.64  6.30±2.04  5.86±2.42 |
The majority of wholesalers, retailers, and consumers pointed to the lack of cold storage as the cause of fish spoilage. At the same time, processors attributed spoilage to insufficient processing and high moisture after processing. These and other responses, such as insect infestation, were scored as correct responses because they pointed to conditions that favored microbial growth. Only six out of the respondents looked correctly at microorganisms, while none mentioned autolysis. It should also be noted that respondents were assessed based on their responses in the questionnaire survey and not their actual observed practices regarding fish handling and food safety practices.

The gender, age, ethnicity, longevity in the fish business, religion, level of education, and the type of stakeholder within the value chain were used to predict the probability that a stakeholder within the fish value chain would have sufficient food safety knowledge and practices. The binary logistic regression results to assess this association are displayed in Table 6.

Based on responses from the questionnaire survey, the model predicted that retailers were more likely to have sufficient food safety practices than other stakeholders within the value chain. Individuals who were Ewe’s, Krobo’s, and Ga Dangbe (predominantly within the freshwater value chain) were also more likely to engage in good fish handling and food safety practices. Therefore, this suggests that stakeholders within the freshwater value chain were significantly more likely to engage in good fish handling practices than those in the marine fish value chain. However, the odds of this livelihood were notably very small (Odds ratio = 0.03, on average).

Figure 5 compares the percentage of stakeholders who were found to have sufficient knowledge of food safety and fish handling practices to establish a relationship between reported knowledge and practices. It can be observed from Figure 5 that, while almost 90% of retailers in the value chain sufficiently practiced food safety rules concerning fish handling, only 30% of these individuals had sufficient knowledge about food safety. A similar trend was apparent among the processors. An insignificant correlation (rho=0.008, p-value= 0.856 at 95% CI) between knowledge and practices supports the argument that many stakeholders practiced good handling practices without necessarily understanding the importance of their actions.

However, most other studies assessing food safety knowledge and practice reported a different trend, where good knowledge did not always translate to good practices. This examines amplified by the findings of Omenu and Aderoju (2008). Their study on the food safety knowledge and practices of street food vendors in Nigeria revealed that good knowledge of the importance of handwashing did not translate into improved quality handling practices. However, the situation among stakeholders in the Ghanaian fish value is less concerned. The lack of understanding of the bases of their good food safety practices may lead them to use those practices nonchalantly.

### Table 6. Logistic regression predicting the level of knowledge and self-reported food safety practices from stakeholders’ demographic characteristics

| Predictor | B   | Wald chi-square | p-value | Odds ratio |
|-----------|-----|-----------------|---------|------------|
| Gender    | -1.36 | 2.421.12       | 0.29    | 0.86       |
| Age       | 0.682 | 1.61           | 0.00    | 0.21       |
| Ethnicity | -    | 4.76           | 0.41    | *0.000     |
| - Ada     | -1.90 | -3.481.74      | 19.05   | 0.00       |
| - Krobo   | 17.68 | -3.140.00      | 6.42    | 0.09       |
| - Ewe     | -2.57 | -3.603.95      | 24.71   | 0.05       |
| Longevity | -0.79 | 0.33           | 0.50    | 0.08       |
| Religion  | -1.04 | -1.124.03      | 0.28    | 0.05       |
| Education | -1.29 | 0.35           | 0.84    | 0.36       |
| Stakeholder | -1.65 | -2.743.18    | 10.34   | 0.00       |

Note: *significant at p-value <0.05; Know= food safety knowledge; Prac= fish handling practices

### Figure 5. Relationship between the knowledge and practices of some stakeholders in the fish value chain

**Fish losses due to spoilage in the artisanal fish value chain**

Stakeholders in both the marine and freshwater value chains reported that they never really considered fish spoil, especially with tilapia, catfish, and sardineillas. According to most, fish only loses its freshness and is useful as food even when it looks rotten. Tilapia and catfish were, for example, salted and sun-dried into “momone” or “lonshala.” Lonshal fetches a much lower price than in the fresh state. Again, lonshala is primarily intended for flavoring and used in tiny quantities during cooking. The fish, therefore, ceased to be a primary source of protein in the diet once it lost its freshness before processing. Stakeholders thus estimated their losses based on fish they had to devalue because they were no longer considered fresh by consumers and customers.

Figure 6 depicts the levels of fish losses due to spoilage experienced by the different stakeholders along the value chain.
Figure 6. Estimated fish losses along the fish value chain

Table 7. Stakeholders’ responses to using fish no longer considered fresh

| Use of “spoilt” fish                  | Number of stakeholders (%) |
|--------------------------------------|----------------------------|
|                                       | Wholesaler/Processor/Consumer/retailer |
| Discarded                            | 1 (1.4)                   | 13 (17.8) | 59 (80.8) |
| Modified to lonskala/momone          | 18 (66.7)                 | 0 (0)     | 9 (33.3)  |
| Animal feed                          | 1 (14.3)                  | 0 (0)     | 6 (85.7)  |
| Sold to consumers as “fresh.”        | 1 (100)                   | 0 (0)     | 0 (0)     |
| Processed as originally intended     | 0 (0)                     | 7 (87.5)  | 1 (12.5)  |
| Returned to purchase point           | 0 (0)                     | 0 (0)     | 2 (100)   |
| Consumed Regardless                   | 0 (0)                     | 0 (0)     | 2 (100)   |
| Never experience spoilage            | 19 (23.8)                 | 20 (25.0) | 41 (51.3) |

Spoilage was classified in this study as low when the losses ranged between zero and 34%. The losses were considered high when as much as 35 to 100% of the fish were lost. Consumers understandably suffered the greatest amounts of losses, sometimes losing up to 100 % of all fish bought, owing to the fact that they were at the very end of the value chain. They also attributed these high losses to Ghana’s reliable electrical power supply. For consumers, the cause of action for fish that had lost their freshness was to discard or use the spoilt fish as animal feed (refer to Table 7). Fish, therefore, intended for use as the primary protein in meals were lost, and significant economic losses had to be incurred to replace the spoilt fish.

Processors, wholesalers, and retailers rarely experienced high losses due to their ability to transform and add value to fish that had lost their freshness. Processors, for example, reported that they could still process spoilt fish as intended initially (without downgrading it to momone) because they believed their processing methods could render the spoilt fish safe for consumption. Fishers experienced minimal losses comparatively, most likely because they reported that they always had a ready market at landing.

Figure 7. Stakeholder estimates of fish downgraded, devalued, or discarded due to spoilage

Figure 8. Relationship between insufficient food safety practices and fish losses among stakeholders

Akande and Dei-Ouadi (2010) reported that between ten and thirty percent of the artisanal catches in Ghana were downgraded due to quality deterioration. Similar findings from this study, displayed in Figure 7, showed up to 34 % losses among 37 % of all stakeholders and between 68 to100 % losses among 13 % of stakeholders.

The stakeholders' food safety knowledge and practices within the fish value chain also appeared to play a role in the reported extent of fish losses. A strong association (Pearson chi-square = 16.137, p-value 0.00) was found between the level of knowledge and the estimated fish losses. This suggested that the level of food safety knowledge of a stakeholder can reliably predict the extent of their fish losses.

Presented in Figure 8, is a graph illustrating the relationship between the extent of fish losses and food safety practice.

Microbiological quality and safety of fish at different stages of the artisanal fish value chain

Salmonella spp., Vibrio parahaemolyticus, and Vibrio cholerae were not detected in any of the samples tested. Ikutegbe and Sikoki (2014) also reported the absence of
this organism, although their study focused only on dried-smoked fish samples. All the suspect *Vibrio* colonies were confirmed as *Aeromonas sobria* by API 20E, with a prevalence rate of 4.8%. Table 8 lists the incidence of some of the pathogenic and spoilage organisms detected in raw and processed fish samples.

The absence of *Salmonella* spp., *Vibrio parahaemolyticus*, and *Vibrio cholerae* on the tested fish samples is of key importance given the significant public health threats they are capable of. However, the presence of *Aeromonas sobria* on both raw and processed fish samples is very troubling. This organism is a pathogen that can cause foodborne gastroenteritis in humans and extraintestinal symptoms such as septicemia, meningitis and endocarditis, and osteomyelitis. It is especially dangerous in immuno-compromised individuals. This organism had been previously isolated from fish by Boari et al. (2007) and Sikoki (2014) who isolated the organism on catfish and tilapia even at refrigerated temperatures. *Aeromonas* spp. have also been implicated in the spoilage of fish (Gram and Dalgaard 2002) because of their ability to produce enzymes such as lipases and proteases.

*Proteus* spp. was detected at a prevalence of 55% in all the raw samples and up to 66.7% in the processed samples. This organism is capable of decarboxylating histidine into histamine, produces H2S, and is therefore capable of causing fish spoilage. It is also an opportunistic pathogen that can infect immuno-compromised individuals. Several studies have implicated pseudomonas species as the dominant bacterium in fish spoilage (Tryfinopoulou et al., 2007; Popovic et al., 2010; Ikutegbe and Sikoki 2014; Viji et al. 2014). However, in this study, the dominant spoilage organisms detected in all fishes at different value chain stages appeared to be *Proteus mirabilis* and *Proteus vulgaris* (Tables 9-10). Akoachere et al. (2009) also isolated *Proteus vulgaris* and *Proteus penneri* in fish sourced from the coastal waters of Cameroun, a country with similar climatic and socio-economic conditions as Ghana.

The total mesophilic counts determined for fresh catfish from fishers and wholesalers were consistently below the maximum allowable limit of 7 Log cfu/g set by the International Commission on Microbial Specifications for Foods (ICMSF), which initially suggested good overall hygienic quality. However, raw tilapia intended for grilling was found to contain unacceptable counts of 7.96±0.68 Log cfu/g consistently. However, this value did not significantly vary from other fresh tilapia and fresh sardinella samples, as presented in Table 9. Erkan and Ozden (2008) assessed the quality of sardines stored on ice in Turkey reported mesophilic bacteria counts of 3.8 to 4 log cfu/g on the first day of storage and up to 6 log cfu/g after nine days of storage. These results were consistent with the findings of this study.

The microbiological quality of the processed fish samples also appeared initially to be good, given the generally low counts of total mesophilic bacteria and total counts of yeasts and molds. A study by Ikutegbe and Sikoki (2014) followed the counts of heterotrophic fungi on smoked fish sourced from retail markets in Nigeria also reported counts of up to 3.40 log cfu/g in smoked fish stored for three weeks or less. Retailers in Ghana kept smoked fish for more extended periods, up to 6 months. Counts for fungi (yeasts and molds) on dried fish samples in this study were generally lower in comparison.

Tables 9-10, however, present evidence to suggest issues with the safety of fish on the Ghanaian market. Essential quality and safety indicator pathogens are detected at different value chain stages. To begin with, *Listeria* spp. They are discovered at a prevalence of 11% in raw tilapia and catfish and up to 66.7% in fermented fish samples. This is of great concern given the public health threat posed by this organism (Bomfeh et al., 2015). The microorganism was also present in salted-dry fish (“koobi”) and smoked-dry fish.

Table 8. Prevalence of some pathogenic and spoilage organisms on raw and processed Tilapia, catfish and sardinella

| Microorganisms       | Raw     | Smoked  | Salted  | Fried   | Dried   | Grilled  | Fermented |
|----------------------|---------|---------|---------|---------|---------|----------|-----------|
| *Salmonella* spp.    | 0 (0/27)| 0 (0/17)| 0 (0/6) | 0 (0/5) | 0 (0/3) | 0 (0/3)  | 0 (0/3)   |
| *Vibrio* spp.        | 0 (0/27)| 0 (0/17)| 0 (0/6) | 0 (0/5) | 0 (0/3) | 0 (0/3)  | 0 (0/3)   |
| *Listeria* spp.      | 11 (3/27)| 23.5 (4/17)| 33.3 (2/6) | 0 (0/5) | 0 (0/3) | 0 (0/3)  | 66.7 (2/3) |
| *Proteus* spp.       | 55.5 (15/27)| 52.9 (9/17)| 33.3 (2/6) | 40 (2/5) | 66.7 (2/3) | 0 (0/3)  | 66.7 (2/3) |
| *Staphylococcus aureus* | 29.6 (8/27)| 17.6 (3/17)| 16.7 (1/6) | 0 (0/5) | 0 (0/3) | 0 (0/3)  | 0 (0/3)   |
| *Klebsiella* spp.    | 48.1 (13/27)| 11.8 (2/17)| 16.7 (1/6) | 0 (0/5) | 0 (0/5) | 0 (0/3)  | 0 (0/3)   |
| *Aeromonas* spp.     | 7.4 (2/27) | 5.9 (1/17)| 0 (0/6) | 0 (0/5) | 0 (0/3) | 0 (0/3)  | 0 (0/3)   |
| *Pseudomonas* spp.   | 11.1 (3/27)| 0 (0/17)| 0 (0/6) | 0 (0/5) | 0 (0/3) | 0 (0/3)  | 0 (0/3)   |
| *Escherichia coli*   | 7.4 (2/27) | 0 (0/17)| 0 (0/6) | 33.3 (1/3) | 0 (0/3) | 33.3 (1/3) |           |
| *Clostridium perfringens* | 77.8 (21/27)| 0 (0/17)| 33.3 (2/6) | 50.0 (3/6) | 100.0 (3/3) | 0 (0/3)  | 0 (0/3)   |
Table 9. Microbial quality of raw, unprocessed fish at different stages of the artisanal fish value chain

| Stakeholder   | Fish species | Product    | n   | Food safety indicators (Log cfu/g) | Food spoilage indicator (Log cfu/g) | Food hygiene indicators (Log cfu/g) | Overall quality indicator (Log cfu/g) | Physico-chemical properties |
|---------------|--------------|------------|-----|-----------------------------------|-------------------------------------|------------------------------------|-------------------------------------|-----------------------------|
| Fishers       | Tilapia      | Fresh fish | 3   | 3.71±0.68a                        | 2.98±0.57a                          | 5.53±0.25ab                        | 29.32±0.37a                       | 7.26±0.17a                  |
|               | Catfish      | Fresh fish | 2   | 2.83±0.22ab                       | 3.70±0.95ab                         | 3.18±0.03a                        | 29.25±0.21a                       | 7.60±0.19a                  |
| Fishers       | Sardineella  | Fresh fish | 3   | 2.70±0.11ab                       | 2.12±0.28a                         | 5.89±0.09ab                        | 26.68±3.09a                       | 7.42±0.15a                  |
| Wholesaler    | Tilapia      | Fresh fish | 3   | 2.31±1.28ab                       | 2.31±1.28a                         | 5.52±2.80ab                        | 19.23±7.89a                       | 6.50±0.13c                  |
|               | Catfish      | Fresh fish | 2   | ND                                | ND                                  | ND                                 | 27.70±0.45a                       | 7.16±0.54a                  |
| Retailer      | Tilapia      | Fresh fish | 3   | 2.83±0.22ab                       | 5.54±2.09b                         | 6.28±0.20ab                        | 15.00±12.45a                      | -                           |
|               | Sardineella  | Fresh fish | 3   | 2.43±0.02ab                       | 2.32±1.19a                         | 5.46±0.36ab                        | 26.00±2.00a                       | -                           |
| Processor     | Tilapia      | Raw fish   | 2   | ND                                | ND                                  | ND                                 | 32.30±3.50a                       | 7.79±0.16ab                  |
| (Fermented)   | Catfish      | Raw fish   | 2   | 2.96±0.39ab                       | 3.52±2.7ab                         | 4.96±0.90ab                        | 30.24±3.53                       | 7.79±0.16ab                  |
| (Grilled)     | Tilapia      | Raw fish   | 2   | 2.71±1.14ab                       | 2.96±0.68a                         | 5.40±1.91a                         | 29.95±3.98a                       | 8.09±0.10b                  |

Note: Values in the same column with different superscripts are significantly different at α=0.05. Abbreviations: A= absent; P= present; PM= Proteus mirabilis; PV= Proteus vulgaris; ND= not detected; Temp: temperature. Lis: Listeria spp.; Kle: Klebsiella spp.; CI perf: Clostridium perfringens; F. coli: faecal coliforms; S. aur.: Staphylococcus aureus; TMC: Total mesophilic count

Table 10. Microbiological quality of processed fish at different stages of the artisanal fish value chain

| Stakeholder   | Fish species | Product       | n   | Food safety indicators (Log cfu/g) | Food spoilage indicator (Log cfu/g) | Food hygiene indicators (Log cfu/g) | Overall quality indicator (Log cfu/g) | Physico-chemical properties |
|---------------|--------------|---------------|-----|-----------------------------------|-------------------------------------|------------------------------------|-------------------------------------|-----------------------------|
| Processor     | Tilapia      | Fermented2    | A   | 2.08±0.14b                        | 2.46±0.00b                          | 4.61±0.10b                         | 2.36±0.00ab                        | 31.95±4.88a                |
|               | Catfish      | Fermented2    | P   | 1.54±0.09b                        | 1.75±1.06b                         | 2.97±0.01b                         | ND                                 | 35.33±2.00a                |
| Processor     | Tilapia      | Grilled       | A   | 1.73±0.70b                        | 5.37±3.79                          | 3.33±0.90ab                        | 37.46±14.23b                      | -                           |
|               | Tilapia      | Smoked        | A   | ND                                | ND                                  | ND                                 | 50.65±21.85b                      | -                           |
| Retailer      | Tilapia      | Smoked        | P   | ND                                | ND                                  | ND                                 | 34.50±4.88a                       | -                           |
| Processor     | Catfish      | Smoked        | P   | 2.39±1.96b                        | 1.93±0.21b                         | 2.24±1.75ab                        | 82.56±2.00b                       | -                           |
| Retailer      | Catfish      | Smoked        | P   | ND                                | ND                                  | ND                                 | 28.27±2.00a                       | -                           |
| Processor     | Sardineella  | Smoked        | A   | ND                                | ND                                  | ND                                 | 28.73±2.00a                       | 6.46±0.03c                |
| Retailer      | Sardineella  | Smoked        | P   | 1.85±0.85b                        | 4.67±1.63b                         | 2.32±1.32ab                        | 31.57±2.61a                       | 6.76±0.35bc               |
| Processor     | Tilapia      | Fried         | A   | ND                                | ND                                  | ND                                 | 34.27±3.03a                       | 7.38±0.10a                |
| Processor     | Catfish      | Fried         | A   | ND                                | ND                                  | ND                                 | 34.27±3.03a                       | 7.58±0.10a                |
| Processor     | Tilapia      | Salted        | A   | 1.75±1.29b                        | 2.17±1.65b                         | 2.66±1.53b                         | 32.68±2.85a                       | 7.47±0.10a                |
| Retail        | Tilapia      | Salted        | A   | ND                                | ND                                  | ND                                 | 28.60±2.00a                       | 7.05±0.00a                |
| Processor     | Sardineella  | Dried         | A   | 3.88±2.54b                        | 3.56±2.93b                         | 4.10±0.37b                         | 31.90±2.00a                       | 7.21±0.10a                |

Note: Values in the same column with different superscripts are significantly different at α=0.05. Abbreviations: A= absent; P= present; ND= not detected; PM= Proteus mirabilis; PV= Proteus vulgaris; Temp: Temperature. Lis: Listeria spp.; Kle: Klebsiella spp.; CI perf: Clostridium perfringens; F. coli: faecal coliforms; S. aur.: Staphylococcus aureus; TMC: Total mesophilic count
A study by Tano-Debrah et al. (2011) attributed the occurrence of Listeria on fermented fish, mainly to post-process contamination. Samples in that study were also collected in James Town and some informal fish markets in Accra, and detectable Listeria monocytogenes on salted-dry and sun-dried tilapia and herrings were reported. Evidence presented also suggested that salting and drying methods used by processors could not adequately control the organism.

However, the high incidence of Listeria on fermented fish in this study was more likely attributable to the opportunities available for proliferation from landing until the start of fermentation. These included consistently high storage temperatures (Table 9) and poor hygienic conditions and handling practices reported from landing until the commencement of fermentation. Unlike the other processing methods, Fermentation was typically carried out as a means to salvage fish that has lost its freshness or fish that may be considered spoiled by consumers. A review of studies on African fermented fishes by El Sheik and Surendran (2014) similarly reported safety issues related to Clostridium, Salmonella, and aflatoxin contamination in momone and other fermented fish products.

It was also noteworthy that the aerobic mesophilic counts of raw tilapia sampled from the grill-processors were not significantly reduced in the final grilled tilapia samples. A similar trend was observed between fresh samples of tilapia and catfish intended for fermentation and salting and their flast processed equivalents. There was also a remarkable persistence of other pathogenic microorganisms like Staphylococcus aureus and Klebsiella pneumonia on smoked and salted fish and the presence of Clostridium perfringens on salted tilapia fried tilapia and sun-dried sardinella samples. It was more plausible to attribute this observation to post-processing contamination rather than the inadequacy of processing methods, owing to poor hygienic conditions of storage and handling reported among stakeholders within the fish value chain. However, questions about the adequacy of processes such as sun drying, salting, and smoking in ensuring safety could not be discounted entirely. Poor hygienic conditions prevailing at most processing sites, in addition to evidence from studies reporting on the poor quality of salt, wash water, and drying temperatures, could very well account for the high incidence of pathogenic and spoilage organisms observed in this study (El Sheik and Surendran 2014). Some of the hygiene issues observed at processing sites included open defecation close to processing areas, presence of livestock, drying of fish directly on the ground or close to the ground, and exposure of fish to the elements, especially during sun drying.

Also of significance was detecting Clostridium perfringens in all the raw fish samples tested. Clostridium perfringens has been implicated as the etiological agent in many food poisoning outbreaks, and its presence on food samples indicates sewerage contamination (Lalitha and Surendran 2003). Therefore, it was not surprising that there were also high counts of fecal coliforms on both the fresh and processed fish samples. The shores of James Town have long been a dumping site of sewerage for the city of Accra, and poor fishing communities along the Volta lake have also been known to dispose of fecal matter into the Lake (Awuah and Abrokwa 2008). The high loads of Clostridium perfringens and fecal coliforms on landed fish from these sources, coupled with the poor handling practices documented in this study, may very likely ensure survival and proliferation of the microorganism even under the dehydrating conditions of processing.

In a similar study, Lalitha and Surendran (2003) reported a 22% incidence rate of C. perfringens in fish and shellfish sampled in Kerala, India. Their study reported the detection of C. perfringens at every stage of the value chain from which the samples were sourced. This was especially the case of the tilapia samples in their study- The microorganism was detected in 94% of all unprocessed raw samples tested (17 out of 18), with counts significantly higher in the raw fish at landing. El-Shorbagy, Reda, and Mona (2012) also reported a prevalence of 57.1% in 56 processed samples and 59% in 57 samples. These researchers also found the microbe in three types of salted sardines but found none in canned fish.

Another important fish quality and safety indicator detected in samples from this study was Escherichia coli. Two diarrhoeagenic E. coli (E. coli (2) 0146 and E. coli (4) 027) were isolated from fresh catfish from fishers and fermented catfish from processors, respectively. The former is an enteropathogenic (EPEC) strain, while the latter is an enterotoxigenic (ETEC) strain of E. coli. Both have been implicated in acute and persistent watery diarrhea in children primarily between the ages of 6 months to 3 years (Warrell et al., 2003). ETEC can particularly cause diarrhea in all ages because of its ability to mimic clinical symptoms of diarrhea. Costa (2013) concluded that the presence of these organisms on the fish samples could indicate the existence of other enteric pathogens and recommended adequate processing before consumption.

Escherichia coli (4) 0148 detected on all the sun-dried sardinella samples were also ETEC strains. This finding is of particular importance because, more often than not, dried sardinellas are consumed without further heat processing in Ghana. They are usually made into powder and used to increase the protein content of complementary feed given to children. This is of obvious concern given the public health implications of ETEC. The survival of ETEC in the otherwise unfriendly conditions of low water activity in the dry fish could be the result of the drying methods employed in processing the sardinellas.

Klebsiella pneumoniae was another fish safety indicator pathogen detected in some samples. It is an opportunistic pathogen that can cause nosocomial infections of the respiratory tract, urinary tract, and blood, especially in children immuno-compromised by a diarrhoeal infection.

Also, the different stakeholders’ temperatures are used in fish storage in all three value chains. The temperatures were less than ideal and may very well account for the incidence of the spoilage and pathogenic organisms observed. The temperature at the landing of all the fish species ranged from 26°C to 29°C. These values did not vary significantly at the next stage of the fresh fish value chain, which involved retailers and wholesalers. The high
average temperatures recorded also appeared to contradict reports by the majority (85%) of wholesalers and retailers who claimed to keep their fish on ice during the sale period.

The disparity between the reported use of ice to reduce the temperature of the fish and the actual recorded temperatures could, however, be explained by the fact that the amount of ice used was insufficient to affect cooling to desirable temperatures. Again, the Styrofoam containers used by stakeholders in the fresh fish value chain, and the wooden boxes with sack lining used in the marine fish value chain, did not provide enough insulation to maintain cold temperatures. The recorded temperatures for the processed fish samples, especially in the freshwater fish value chain, were exceptionally high (50 to 85°C) and significantly different from other fresh and processed fish temperatures because they were sampled a few minutes after processing.

However, the pH of the fish measured at the landing and different stages of the value chain appeared to conform to trends reported in similar studies. The general pH of fresh tropical fish muscle reported by Susanto et al. (2011) ranged from 6.0 to 7.3 on the first day of capture and from 6.8 to 8.2 by the second day when kept at ambient temperature. In this study, the pH at landing (from fishers) ranged from 7.2 to 7.8 for the freshwater fishes and between 7.3 and 7.6 for the marine fish. These values significantly decreased at the wholesale stage in the tilapia value chain. Because the same batch of fish was not followed from fishers to wholesalers, it was impossible to accurately assume that the observed significant increase in acidity from one stage in the value chain to the next was due to microbial or autolytic activity. High glycogen levels in the fish before capture may account for the increased acidity because of the resultant accumulation of lactic acid.

A significant rise in alkalinity in raw catfish and tilapia intended for fermentation and salting was observed from samples sourced from processors. Therefore, the stakeholders considered such fish no longer fresh and transformed into “Momone” and “Koobi.” A rise in fish muscle alkalinity with increasing storage time was also observed in a study by Erkan and Ozden (2008). These researchers attributed the pH increases to the accumulation of alkaline compounds such as ammonia, mainly derived from microbial action. Vijji et al. (2014) also supported this theory but warned that pH was a poor quality indicator of fish quality.

Compared to the freshwater value chain, fewer pathogens were isolated from the marine fish value chain. Sardinellas, which were to be sold fresh to consumers, were also potentially stored for up to two weeks by retailers. However, this did not imply that the quality of the marine fish was better than freshwater fishes, evidenced by the high unacceptable total mesophilic counts observed in raw tilapia samples.

**Figure 9.** Association between food safety practices and the actual microbial quality of Artisanal fish

**Association between stakeholder knowledge and practices and the actual microbial quality of artisanal fish**

It is evident from Figure 9 that pathogens and spoilage organisms could be found at almost every stage of the value chain where poor handling practices by stakeholders were reported. The exception was with wholesalers and retailers of fresh, unprocessed fish, who generally said sufficient handling practices but still had sick quality fish. This deviation from the trend was clearly a result of insufficient cooling temperatures and temperature abuse during storage. Temperature abuse was observed throughout the value chain and is, without question, a significant reason for the poor quality of fish found in this study.

Another possible contributor to the poor quality of fish observed at the retail and wholesale stage of the fresh fish value chain could be the existing traditional laws that place certain restrictions on the uninhibited fish flow on the artisanal value chain. Fishing in James Town on Tuesdays, for example, was strictly taboo. Consequently, the women who retailed fresh or raw fish in the local fish market were also not allowed to sell their fish on Tuesdays. Although crucial in ensuring the sustainability of fisheries, this cultural practice also implied that fresh fish remained longer (up to two weeks) at the retail stage of the value chain.
chain. This represented a classic case in which a stakeholder practice that was not directly related to food safety may have implications for the poor quality of fish observed at the retail stage.

Figure 9 also shows potential opportunities for recontamination of processed fish in the artisanal fish value chain. Here again, poor handling practices by processors themselves and retailers of processed fish may cause the poor quality of processed fish to reach the consumer. This is of particular concern given that fish at these stages of the value chain (processor and retailer) led directly to the consumer, who may, in all likelihood, consume this fish without further processing.

The high reported fish losses due to spoilage were also corroborated by detecting various spoilage microorganisms, particularly Proteus spp. At different stages of the value chain, Figure 9 indicates the risk of fish spoilage, especially with fish reaching wholesalers and retailers from fishers, at the actual retail and wholesale point, and during processing before entering the consumer, the highest incidence of spoilage reported by wholesalers and retailers. The few processors who reported losses said that waste occurred mainly before processing during pre-process operations such as spicing before grilling or sun-drying before hot-smoking. This is again confirmed during microbiological testing as spoilage organisms isolated at these exact points in the value chain.

The link between the food safety knowledge and practices of stakeholders and the actual microbiological quality of the fish thus becomes visible. Poor food safety knowledge and handling practices of stakeholders have clear implications on the final microbiological quality of fishes handled by these stakeholders.

Food security implications

Given the findings of this study, a valid argument can be made about a potential threat to food security. To begin with, the significant economic implications of high reported fish losses are undeniable. Although one may argue that much of this loss is salvaged through fermentation processing, economic losses are still incurred because fermented fish fetches a considerably lower price than fresh fish. It no longer serves its purpose as a primary protein in a meal. Fermented fish is typically consumed in very small quantities as a condiment for soups and sauces and is therefore not consumed in sufficient quantities to represent a significant source of protein. Ikutegbe and Sikoki (2014) also reported a decline in fish protein levels, increasing microbial load and storage time. Again, the high quantities of salt used in fermentation render the fish quite unhealthy when frequently used in meals (Table 11).

The majority (61%) of consumers in this study reported that they used meat as an alternate source of protein when fish was unavailable. They, however, stated that meat was more expensive than fish and therefore had to spend more money on food when fish was unavailable. Up to 80% of consumers also chose fish as their primary source of protein because they believed it was healthier than other protein sources. The majority (76%) also trusted the safety of the fish on the local market, a matter of concern, given the probability that most of these consumers may not take the necessary precautions in processing their fish because they trust it to be safe.

Regarding safety, the poor microbiological quality of fish from artisanal sources could contribute to the high disease burden among the most vulnerable groups. Fish has been implicated in several outbreaks of food-borne infections. It is a potential vehicle for food-borne illnesses such as cholera, listeriosis, salmonellosis, and others (Popovic et al., 2010; Costa 2013; Akoachere et al. 2009). In Ghana, diarrheal diseases are among the top three causes of death among children under five. According to a WHO/UNICEF report, 55,000 children under five died from diarrhea in 2008 alone (Wardlaw et al., 2010). Diarrhoeagenic microorganisms such as E. coli, Aeromonas sobria, and Clostridium perfringens detected on ready-to-eat fish given as complementary feed to children may very well contribute to the high incidence of diarrhea in Ghanaian children under five.

From the nutritional, safety, and an economic point of view, the food security of the many Ghanaians who depend on artisanal fish as a primary source of protein and livelihood may be under reasonable threat.

Table 11. Consumer responses to questions on food security about fish availability and safety

| Tested components of food security | Consumer questions                                                                 | % Responses of consumers |
|-----------------------------------|------------------------------------------------------------------------------------|--------------------------|
| Affordability                     | Are you able to afford fish every time?                                            | 73 27 19                 |
|                                   | What are your alternate sources of protein when fish is unavailable?               |                          |
| Meat                              | 61 - -                                                                            |                          |
| Eggs                              | 23 - -                                                                            |                          |
| Legumes                           | 3 - -                                                                             |                          |
| Mushrooms                         | 8 - -                                                                             |                          |
| Crab/snails                       | 2.5 - -                                                                           |                          |
| Vegetables                        | 2.5 - -                                                                           |                          |
| Is meat more expensive than fish? | 55 40 5                                                                           |                          |
| availability                      | Are you able to get fish every time you want it (all year round)?                  | 81 19 -                  |
| Health/nutrition                  | What is your reason for choosing fish as your primary source of protein?           |                          |
| Health                            | 42 - -                                                                            |                          |
| Fish is more nutritious           | 38 - -                                                                            |                          |
| Fish is cheaper                   | 8 - -                                                                             |                          |
| Taste                             | 12 - -                                                                            |                          |
| Are there adverse health effects  | 48 12 40                                                                          |                          |
| associated with eating unwholesome fish? |                                              |                          |
| Do you think fish sold on the local market is safe for human consumption? | 76 22 2                                                                           |
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