Coliform and *Escherechia Coli* levels in the Yellow Fin Tuna (*Thunnus albacares*) landed at the Dixcove beach in Ghana


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**ABSTRACT**

Even though enough education has been given to the artisanal fishermen along the coast of Ghana by the Department of Fisheries on the need to keep the fish landing sites clean and also to place landed fishes on clean polythene mats, yet it appears this has not made the desired impact (Anon, 1995). Two separate studies were conducted in May and June 2009 to determine the coliform levels of Yellowfin tuna (*Thunnus albacares*) on freshly landed and processed (smoked) forms at the Dixcove beach. On weekly basis a composite fish sample either fresh or smoked, consisting of 10 g each of gills, gut and muscle were blended to form a composite sample which was used to prepare serial dilutions in 250 mls of distilled water. The solutions of both fresh and smoked tuna were poured into a conical flask and plugged with cotton wool and further covered with aluminium foil to prevent further contamination. Serial dilutions of both fresh and smoked fish were done accordingly by taking 18 mls of distilled water and adding 2 mls of the composite initially and further dilutions were done from the initial one by following the same method. The resultant solutions with the microorganisms were cultured using Plate Count Agar (PCA) and MacConkey and following the method adopted by Harrigan et al., 1966, the colonies on the petri dishes on which the microorganisms were identified and counted in colony forming units. A range of (1.8-2.6) x 10^6 CFU/ml was obtained for the total viable counts for fresh fish and this is higher than the standard for The Ghana Standard Board (GSB) (1x10^6). In the same way a range of (6.5-9.6) x10^2 CFU/ml was obtained for Escherechia coli (E. coli) colonies and this was also above the GSB value of 4x10^1. This shows higher levels of fresh fish contamination at the landing site. The results for the processed fish samples showed significant reduction in the colony counts as well as E. coli levels compared to results for fresh fish samples indicating that processing (smoking) had an impact on reducing the levels of contamination in the processed fish. The total viable counts in the smoked fish ranged from (1.1-2.6)x10^5 which is less than the GSB value of 1x10^5. For the smoked fish E. coli level ranged between (4-8)x10^1 which is slightly above the GSB acceptable level of 4x10^1. The experimental results suggested that the total viable counts
and E. coli levels of the freshly landed Yellowfin tuna by the artisanal fisheries at the Dixcove beach fell below the acceptable standards by the Ghana Standards board(GSB) and therefore not safe for consumption and export and therefore the need to improve upon their method of landing and handling fish at the landing site. The smoked fish were almost within the acceptable level by the GSB. Smoking therefore is effective in reducing the coliform level in the fish but more needs to be done to improve the quality of processed fish so as to reduce the level of bacteria contamination to ensure that quality fish is sold and eaten in Ghana and can also be exported without difficulty from the artisanal fisheries. It is recommended that there is the need to do continuous monitoring of fish landing sites to educate the artisanal fisherfolk on the need to improve their method of landing and handling fish landed so as to minimize level of contamination by bacteria and other pathogenic agents.

INTRODUCTION

Even though fish protein is one of the most available proteins for consumption by Ghanaians and may be harvested in both freshwater and marine habitats, most of the fish are obtained from the sea (Koranteng et al., 1993). The major challenge faced by most places in the world including Ghana, is the unhygienic environmental conditions in which the fish finds itself after capture before it comes to the table for consumption. Methods used in hauling or processing fish are likely to contaminate the fish with pathogens (Akrofi, 2002). Again contaminated fish may lead to serious losses by traders involved in the fishing industry. Fish contaminated by pathogens have serious health consequences and are responsible for some of the deaths registered in Ghana and other parts of the world (Mensah et al., 2002; WHO, 2002 and Scott et al., 2007). Commercial fishing vessels all over the world that fish tuna and other fishes are required to deliver safe fish, free from any contamination to their consumers (Taylor, S.L., 1983; EEC, 1990). WHO and FAO and other recognized bodies such as ICMSF have established safe standards for fish and its related products for consumption by humans (WHO, 2001; FAO, 1989; ICMSF, 1988). Almost all countries require some form of safe standards to be conformed to before a given country would be allowed to export fish and its products into that country (FAO, 1989). One of the methods used to test for safe standards of fish and their related products is to test for the coliform bacteria, histamine and mercury level of accumulation before export (Food Safety Act., 1990). In Ghana, the Ghana Standards Board (GSB) has established safe standards for fish and its products for consumption aimed at ensuring that fish and its products consumed should be wholesome. Even though these safe standards have been established in Ghana, very little or no steps are taken to enforce these standards at the artisanal level and hence no serious safety measures are ensured for the benefit of consumers. The fisheries officers placed at the landing beaches have no power to prosecute or arrest people who are not conforming to these standards but can only educate the fisherfolk.

In Ghana scanty literature exists on coliform levels of landed and processed fish. A research conducted by Food Research Institute of Council for Scientific and Industrial Research (CSIR) in partnership with the Natural Resource Institute of Britain on Ghanaian street vendor foods in the street of Accra (Mensah et al., 1999; Mensah et al., 2002), indicated that the Escherichia coli, Clostridium perfringens, Staphylococcus aureus, Bacillus cereus were present. The presence of Salmonella, a deadly poisoning bacteria was also confirmed. In similar studies, Essuman (2001)
also detected high levels of pathogenic organisms in some of the prepared food samples for sale on the Ghanaian market he analysed. The presence of these organisms could either be traced to poor handling of the food during processing or from the sources where these products were obtained such as the fish landing sites or markets (Akrofi, 2002). Recently Obodai et al. (2010) reported depuration reduced bacterial and fungal colonies in oysters (Crassotrea tulipa).

Due to scanty literature on levels of bacterial contamination of landed fishes by artisanal fishermen in Ghana, a study was conducted to examine the level of contamination in both freshly landed and processed tuna fish (smoked fish) at one of the busiest artisanal fish landing sites in Ghana, Dixcove in the Western Region of Ghana. Yellowfin tuna was chosen for this study because it is one of the most preferred tuna for consumption and is highly priced (Pers comm.). Dixcove was chosen for the study because it is one of the most important fishlanding sites for tuna by the artisanal fishermen (Debrah, 2000 and Ofori-Danson et al., 2003).

The main objective was to determine the level of contamination of bacteria on landed and smoked yellow fin tuna at Dixcove beach.

The specific objectives were:
To determine the E. coli level in both freshly landed and smoked forms of the fish.
To determine the coliform level of both freshly landed and smoked forms of the fish.
To determine whether the E. coli and coliform levels were within the standards set by the Ghana Standards board.
To determine whether the education on the need to handle landed fish hygienically by the Fisheries Department of the Ministry of Food and Agriculture in Ghana has had the desired impact on the fisherfolk.

The results were to be used to educate the Ghanaian community at the coastal areas on the levels of contamination of bacteria of both landed and processed tuna fish and to educate the artisanal fisherfolk on the need to keep the beaches clean and improve on their methods of handling and landing fish to reduce levels of fish contamination by bacteria.

MATERIALS AND METHODS

Study Area
Dixcove beach is located in the Western Region of Ghana (4°47'60N, 1°57'0W). The beach is rocky with a small portion being sandy. Fishing and fish processing (smoking, salting and fermenting) are the main occupation of most indigenes of the town. The small sandy portion of the beach is the landing site for the fishermen with an observable deplorable sanitary conditions. People defaecate on the beach and also use some parts of the beach as refuse dump where both solid and liquid wastes probably from processed fish and old fishing nets are disposed.

In this study, special culture media namely MacConkey Agar, and Plate count Agar were prepared and used in culturing the bacteria and the results obtained were compared to recommended standards of the Ghana Standard Board (GSB) to evaluate the risk factors and threats posed to consumers of the Yellow fin Tuna (Thunnus albacores) landed at the Dixcove beach. Sterilization:
Petri dishes were washed with detergent soap, rinsed with tap water, then distilled water and left to dry at room temperature. They were then put in a canister and sterilized in an oven at a temperature of 160 °C for 6 hours. The volumetric flasks, test tubes and measuring cylinder were, also washed with detergent soap, rinsed, dried and stoppered with cotton plugs, and capped with papers and autoclaved at 121 °C for 15 minutes. The pipette tips were wrapped in a grease proof paper and autoclaved at 121 °C for 15 minutes. The plastic rubber bags into which the fish samples were put, were folded singly, wrapped in a grease proof paper then autoclaved at 121 °C for 15 minutes.

**Fresh fish sampling**

Samples of fresh yellow fin tuna (T. albacares) were randomly bought from different fishermen who landed their fish at different locations of the beach. The fresh fish samples were placed in transparent, sterilized (autoclaved) plastic rubber bags, sealed at the open ends and then iced in an iced chest for transport to the laboratory for study. The fish samples were iced to stop the multiplication of bacteria colonies.

**Smoked fish sampling**

Smoked Yellow fin tuna (T. albacares) were bought from 4 different fish processors at different smoking sites or houses. Samples from each site were placed in different clean sterile rubber bags, sealed at the open ends and then placed in a plastic bag for transport to the laboratory for study.

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*Figure 1: Coastal map of Ghana showing sampling site.*
Preparation of media

*Plate count agar*

22.5 g of plate count agar (PCA) were weighed and dissolved in 1 litre of distilled water and then autoclaved at a temperature of 121°C for 15 minutes.

*MacConkey Agar*

40 g of MacConkey agar was suspended in 1 litre of distilled water and heated to boil in order to dissolve completely. It was then sterilized in an autoclave for 15 minutes at 121°C.

Fish sample preparation

A composite sample was prepared for each week’s sample by taking about 10 g each from the gills, gut and the muscle of each fish for both the fresh fish and smoked samples. The resulting sample was blended in 250 ml of distilled water. It was poured into a conical flask and plugged with cotton wool and further covered with aluminium foil to prevent contamination.

Preparation of serial dilution

Three clean dry sterilized test tubes, labelled F1, F2, and F3, a pipette filter, about four (4) dozen pipette tips were employed. Each of the test tubes (F1-F3) was filled with 18 ml distilled water and then 2 mls of the prepared fish sample were pipetted into the test tube F1; it was then gently and thoroughly shaken. 2 ml of the resultant solution in F1 were pipetted into F2, and was thoroughly shaken as the former to mix well. Again 2 mls of the resultant solution in F2 were also pipetted into F3 and shaken gently to mix well. In each case a different pipette tip was used to avoid contamination. For PCA inoculation, the process was done for six test tubes, thus F1-F6. The serial dilution was done separately for fresh and smoked fish samples. The tubes for fresh fish samples were labeled F1-F6 and for the smoked fish samples S1-S6.

Isolation of microorganisms

The MacConkey and Plate Count Agar were melted over a water bath and allowed to cool. About 2 mls of solution were taken from each of the test tubes F1 to F3 and S1 to S3 used for the serial dilution were poured into petri dishes labeled $10^1$, $10^2$, $10^3$ for MacConkey Agar, then F1 to F6 and S1 to S6 for the PCA, resulting in 36 different petri dishes. The cotton wool at the mouth of the conical flask containing the medium was removed and the mouth of the conical flask flamed with spirit lamp. About 10 mls of each medium was poured into three petri dishes containing different concentrations of the sample ($1:10^1$, $1:10^2$, $1:10^3$) and were clearly labelled as such.

The media were allowed to solidify and the petri dishes placed upside down in an incubator at 37°C for 24 hours, after which the colonies were counted.

Colony identification

The colonies on the petri dishes were counted and the colony forming units (CFU) recorded. Colonies growing on the petri dish plates were identified based on the colony morphology and characteristics as well as a biochemical investigation, Harriganet al. (1966).
RESULTS

Table 1 shows that PCA counts for fresh fish sample values ranged between \((1.8-2.3) \times 10^6\) which were relatively higher than the GSB Value of \(1 \times 10^6\) CFU/ml.

For smoked fish, PCA counts showed a range of \((1.1-1.6) \times 10^5\) which were lesser than the GSB value of \(1 \times 10^5\) CFU/ml. This means that smoking reduced total bacteria levels in processed fish to acceptable limits.

For *E. coli*, fresh fish values ranged between \((6.5-9.6) \times 10^2\) which were higher than the GSB value of \(4 \times 10^2\) CFU/ml. For the smoked fish, values ranged between \((4-6) \times 10^1\) compared to the GSB value of \(4 \times 10^1\) and this means that the observed values were slightly above the GSB value.

**Table 1: Mean total viable counts of bacteria on test Tuna samples (cfu/mL)**

<table>
<thead>
<tr>
<th>Period of sampling (Week)</th>
<th>Type of microorganisms</th>
<th>Fresh ((x10^5))</th>
<th>Smoked ((x10^5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Bacteria (PCA)</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>23</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em> (MA)</td>
<td>88 (x10^1)</td>
<td>4(x10^1)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>73</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>65</td>
<td>6</td>
</tr>
</tbody>
</table>

Ghana Standard Board: (Total count: \(1 \times 10^6\)cfu/ml; *E. coli*: \(4 \times 10^2\)cfu/ml)

PCA: Plate Count Agar
MA: MacConkey Agar
CFU – Colony Forming Unit.

In Table 2, results show that fresh fish samples had values for PCA counts around \(2.6 \times 10^6\) which was more than the GSB value of \(1 \times 10^5\) about 3 times the GSB value. For the smoked fish the PCA counts ranged between \((2.4-2.6) \times 10^2\) which was less than the GSB value of \(1 \times 10^5\) and therefore acceptable.

**Table 2: Mean total count of bacteria on test Tuna samples (CFU/ml)**

<table>
<thead>
<tr>
<th>Period of sampling (Week)</th>
<th>Type of microorganisms</th>
<th>Fresh ((x10^5))</th>
<th>Smoked ((x10^5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Bacteria</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>(PCA)</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>NG</td>
<td>26</td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em></td>
<td>85 (x10^1)</td>
<td>8(x10^1)</td>
</tr>
<tr>
<td>2</td>
<td>(MacC. A)</td>
<td>90</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>87</td>
<td>8</td>
</tr>
</tbody>
</table>

Ghana Standard Board (GSB):(Total viable count is: \(1 \times 10^6\) CFU/ml; *E. coli* is \(4 \times 10^2\) CFU/ml for fresh fish samples.

GSB values for Smoked fish are: Total viable counts:- \(1x10^5\) CFU/ml and *E. coli* is \(4x10^1\) CFU/ml.

*NG means no growth of bacteria was observed.
DISCUSSION

From the results obtained, values for fresh fish samples for both PCA and E. coli counts were higher than the recommended values by the Ghana Standard Board. For example, a range of 1.8-2.3 CFU/ml obtained for the total viable count for fresh fish for the four weeks period is more than twice the standards of the GSB which is $1 \times 10^6$ CFU/ml. The same trend was also repeated (see Table 2) when a similar study was carried out for a period of three weeks. For the E. coli counts, a similar pattern was observed in the two separate studies. In Table 1, more than twice the GSB value ($9.6 \times 10^2$ CFU/ml) was obtained compared to the GSB value of $4 \times 10^2$ CFU/ml. However, smoking reduced the coliform levels to the acceptable limits. This is evidenced by values for both the PCA and E. coli counts for smoked fish which fell within the GSB values but with few deviations. See Table 2, where values above GSB ($6-8 \times 10^1$) were recorded for E. coli counts in smoked fish. These values were beyond the acceptable limits and therefore more needs to be done to improve the methods of processing fish especially smoking and the way it is handled during processing. This is to ensure that quality fish is brought to the market to avoid people buying contaminated fish products and also to ensure that the shell life of both processed and freshly landed fish is increased. The results of this study give an indication of the poor insanitary conditions of our landing beaches and therefore there is the need to continue to sensitise our fisherfolk in the artisanal fishery on the need to keep the landing sites or beaches clean and again the need for District Assemblies to providetoilet facilities at the landing beaches to avoid people defaecating at the beaches. Again rules ensuring that fishes caught are not placed on the bare floor should be enforced and offenders punished by being fined by the local authorities. The study revealed that the poor handling of the fresh fish with unwashed hands, dirty clothing of fish processors, landing fish on bare floor instead of on neat plastic mats have all contributed to the high coliform levels encountered. It was observed that a significant amount of time is spent before the landed fish is actually carried from the beach to the house for processing and this time should be reduced since most of the fresh fish landed is poorly frozen or not frozen at all. Reducing the time would help to reduce the bacteria load and hence reduce high levels of E. coli recorded.

Comparing the bacteria colony counts for both freshly landed and smoked fish; it could be observed that there was a great reduction in the number of colonies counted on the smoked fish thus making it relatively safe for consumption than the freshly landed fish.

CONCLUSION

The study has revealed that coliform levels of fresh yellow fin tuna, *Thunnus albacares* landed at the Dixcove beach was relatively high especially the fresh fish and so do not meet the standards of Ghana Standards Board (GSB). However, smoking reduced the level of coliform of yellow fin tuna significantly. This means that the method of handling the fish and packaging should be improved inorder to bring the coliform level to an acceptable standard for consumers.

The high coliform counts for the fish could also be attributed to the poor sanitary conditions that prevailed at the landing and processing sites of the study area and therefore the municipal Assembly, Fisheries Department and Traditional authorities must step up their effort to educate
the fisherfolk and the fish processors to ensure that clean landing and processing sites of fish are maintained to ensure that quality fish are sent to the market for consumption.

It must be emphasized that this situation is not limited to tuna alone but the poor insanitary conditions at the landing beaches especially at the coasts exposes other fishes landed at the same site to a lot of bacteria load and could be potential dangers for spread of diseases and effort must be put in place to avoid this contamination.

REFERENCES