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# SUSTAINABLE FISHERIES MANAGEMENT PROJECT (SFMP)

## Microbiological and PAH Profile of Smoked Sardines In Ghana



THE  
UNIVERSITY  
OF RHODE ISLAND  
GRADUATE SCHOOL  
OF OCEANOGRAPHY



**SNV** SMART  
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## **ACRONYMS**

|       |  |
|-------|--|
| BaP   | Benzo (a) pyrene                                   |
| CSIR  | Centre for Scientific and Industrial Research      |
| EC    | European Commission                                |
| FDA   | Food and Drugs Authority                           |
| FRI   | Food Research Institute                            |
| GDP   | Gross Domestic Product                             |
| GSA   | Ghana Standards Authority                          |
| PAH   | Polycyclic aromatic hydrocarbons                   |
| SFMP  | Sustainable Fisheries Management Project           |
| SNV   | Netherlands Development Organization               |
| USAID | United States Agency for International Development |

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## EXECUTIVE SUMMARY

This assessment was conducted with the assistance of technologists and scientists from CSIR-Food Research Institute, Ghana and Institute of Marine Research, Norway respectively from June to November, 2018. This assessment was requested by the SNV to assess the microbiological safety of small pelagic, the level of PAH in samples of fish smoked with Chorkor, Barrel and Improved Ahotor oven, the carcinogenic and non-carcinogenic risk associated with fish samples smoked with Chorkor, Barrel and Improved Ahotor ovens.

Major findings:

1. For smoked *Sardinella aurita* and *Sardinella maderensis*, *E. coli* and *Staphylococcus aureus* were the only microbes not detected.
2. For fresh *Sardinella aurita* and *Sardinella maderensis*, *Staphylococcus aureus* and *Clostridium perfringes* were the only microbes not detected.
3. Level of BaP in smoked fish samples from Chorkor and Barrel ovens exceeded the European Commission limit of 5 ug/g WW.
4. Level of BAP in smoked fish samples from Ahotor oven through the application of wood ash was lower than the European Commission limit of 5 ug/g WW.
5. Consumers of smoked fish samples from Chorkor and Barrel ovens are vulnerable to carcinogenic and mutagenic disorders.
6. Consumption of smoked fish samples from Ahotor oven (using wood ash on the fat collector) possess no carcinogenic and mutagenic disorders to consumers.



## 1. INTRODUCTION

Ghana is significantly endowed with valuable fish stocks and a strong tradition and culture of fishing comparable to other West African nations. Indeed, the country produces on the average 440,000 tons of fish from its waters each year. Ghana is both an exporter and importer of fish. Over the years, Ghana has exported large volumes of fish (about 46,000 mt) to international markets (MOFAD, 2012). Revenues from the fisheries sector accounts for about US\$1 billion per year, contributing at least 4.5 percent to Ghana's GDP (FAO, 2006).

Fisheries products supply 60 percent of the animal protein consumed in the country and per capita fish consumption is 27 kg per year – more than double the world average (COMHAFAT, 2012). Whether fresh, more often smoked, dried, or even as powder, fish is a critical source of dietary protein and micronutrients such as iron, iodine, zinc, calcium, vitamin A and vitamin B for many communities in rural areas particularly in the poorest regions of Ghana.

The traditional methods of fish processing and preservation comprise smoking, drying, frying, salting and fermenting and in some circumstances a combination of these methods. It had been reported that 95% of fish processed in Ghana is smoked, 3% salted while the remainder is dried, fried or fermented (SMFP, 2015). Unfortunately, fish production in the country is confronted with a number of challenges. It is worthy to note that fish is exposed to microbial and chemical contamination right from the catch, through the processing, storage and final display at various local markets. Seafood is a highly perishable food commodity and one of the major causes of fish spoilage is by microbial contamination (Sumner and Ross, 2002). There are two groups of bacteria that are of public health importance in the seafood industry. These are: (1) those initially present in the seafood (2) those that are introduced in the seafood by improper handling and storage conditions (Ripabelli et al., 2004; Scoglio et al., 2000). Previous researchers have indicated that microbial contaminations in seafood are mostly due to improper storage and handling practices (Ananthalakshmy et al., 1990). Their study has found that microorganisms such as *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus*, *Aeromonas hydrophyla*, and *Clostridium botulium*, *Listeria Monocytogenes*, *Enterobacteriaceae* sp such as *Salmonella*, *Shigella* and *Escherichia coli* are common contaminants in fish. Other species of *Vibrios* such as *Vibrio alginolyticus* and *Vibrio fluvalis* are also introduced in seafood due to improper handling and storage techniques (Bhaskar and Sachindra, 2006). The improper practices during handling, storage and processing of seafood cause pathogens to multiply exponentially under favorable conditions resulting in seafood infections (Al-Harbi and Uddin, 2005). These microbial agents in seafood may result in a serious threat to the seafood industry with a high risk of illness and disease.

Smoking preserves and enhances flavor; however, as the fish gets in contact with the smoke from the firewood, polycyclic aromatic hydrocarbons (PAHs) are formed and deposited on the fish product. PAHs are a class of high lipophilic compounds that comprise of chemical compounds known to be potent carcinogens (Simko, 2002). Structurally, they consist of one or more aromatic rings and their structure is known to influence their toxicity. PAHs formation in smoked foods depend on several variables in the smoking process, including type of smoke generator/stove, type of fuel used, combustion temperature, and degree of smoking (Garcia and Simal, 2005). Other factors include; fat content of fish species and cooking time.

The smoking industry in Ghana is largely unregulated with various oven types, hygienic issues and wood fuels used for smoking; as such the quality of smoked fish varies from place to place. Fish smoked in Ghana is sold in-country with some units exported to other regional markets. Accessing the EU market has however proven difficult as the Union demands adherence to stringent regulations and standards regarding the sourcing, handling and processing of smoked fish. Amongst such standards, is the limit of Polycyclic Aromatic Hydrocarbons in smoked fish. Thus research focused on quantifying the various PAH levels in different fish species of both smoked and fresh fish on the Ghanaian market to be able to provide scientific information and risk exposure to the ordinary consumer. Further analysis into microbiological profiles of fish, both smoked and fresh was carried out by sampling fish from the popular sales points and markets in Ghana to determine their contamination levels. Based on these results and existing information (FAO), SNV in consultation with project partners and national stakeholders aim to develop smoked fish production protocols to reduce contamination levels in smoked fish. Also, smoked, salted and fresh fish samples were found to carry various microorganisms including bacteria, molds and yeasts. This study reiterates the need to intensify education on hygienic and best processing practices of fish.

### **Objectives of the study**

The primary aim of this study was to assess the quality of smoked and fresh small pelagic fishes on the Ghanaian markets.

Specific objectives included:

1. Estimating the levels of some microbiological organisms that threaten the health of consumers in smoked and fresh fishes;
2. Assessing the level of Polyromantic hydrocarbons (PAHs) in smoked fishes;
3. Estimating the vulnerability of consumers to cancer related diseases from consuming smoked fishes.

## 2. LITERATURE REVIEW ON POLYCYCLIC AROMATIC HYDROCARBONS

Generally, PAHs are hydrocarbon compounds which can be found in coal and all oil types, such as crude oil, petroleum, benzene, and diesel. The PAHs structure consist of carbon and hydrogen atoms in the form of two or more fused aromatic (benzene) rings. A benzene ring shares a pair of carbon atoms with another ring (Gary & Petrocelli, 1985). In the purest form, PAHs are flat, solid, and range in appearance from colorless to white or pale yellow-green. In general, PAHs in the environment are produced from incomplete combustion of substances with carbon molecules such as oil, wood, or coal. Other anthropogenic sources include motor vehicles, cooking ovens, and cigarettes. Their structures are in a single plane. Generally, their molecular weight is in the range of 166 to 328. The molecular weight of each molecule depends on its number and position of fused rings and other components. PAHs are crystalline solid with high melting and boiling points but with low vapor pressure and water solubility. Furthermore, they have a high affinity for solid particles, especially with high organic content. Thus, when they reach the aquatic environment, they tend to rapidly adsorb on solid surfaces such as suspended particles and sediments.

Generally, PAHs in linear form are less soluble than angular or condensed form (NRCC, 1983). Polycyclic aromatic hydrocarbons (PAHs) are a class of high lipophilic compounds that comprise a class of chemical compounds known to be potent carcinogens. Due to their carcinogenic activity, PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists. PAHs are present in the environment; in water, air, soil and traces of these substances have been found in various food products. Food can become contaminated during thermal treatments that occur in processes of food preparation and manufacture (drying and smoking) and cooking (roasting, baking, and frying) (Ishizaki et al., 2010). Most PAHs are chemically inert, hydrophobic, and soluble in organic solvents. PAHs are ubiquitous environmental pollutants, resulting from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities they originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, and coal-derived products, with combustion sources predominating (Simko, 2002). Human exposure to PAHs occurs in three ways, inhalation, dermal contact and consumption of contaminated foods.

Diet is the major source of human exposure to PAHs as it accounts for 88 to 98% of such contamination (Farhadian et al., 2011). Processing of food at high temperatures (grilling, roasting, frying and smoking) are major sources generating PAHs. Level as high as 200 $\mu$ g/kg has been found for individual PAH in smoked fish and meat samples. For instance, in barbecued meat, 130 $\mu$ g/kg has been reported whereas the average background values are usually in the range of 0.01 to 1  $\mu$ g/kg in uncooked foods (Guillén et al., 2009). Fish is a rich source of lysine suitable for supplementing high carbohydrate diet. It is a good source of thiamin, riboflavin, vitamins A and D, phosphorous, calcium and iron. It is high in polyunsaturated fatty acids that are important in lowering blood cholesterol level (Al- Jedah et al., 1999).

It is well known that raw meat (from mammals) does not contain appreciable levels of carcinogenic PAHs and no accumulation along the food chain has been observed for these contaminants in animal fat tissue (EFSA, 2008). More controversial is the presence of PAHs in vertebrate fish. According to most reports, due to their ability to rapidly metabolize PAHs, fish generally contain very low PAH concentrations, even when they come from heavily contaminated areas (Oost et al., 2003). Nevertheless, some researchers found high PAH

concentrations in raw fish from contaminated waters (Visciano et al., 2006). Some authors (Akpan et al., 1994) reported benzo pyrene (BaP) concentrations ranging from 5.4 to 44.0  $\mu\text{g kg}^{-1}$  dry weight for raw fish from three (3) Nigerian cities.

More recently, Anyakora et al. (2008) reported BaP concentrations between 1.5 and 10.5  $\mu\text{g kg}^{-1}$  in 4 different fish samples from the Niger delta, a highly contaminated site due to the extensive petroleum production activities. All food processing involving thermal treatments at high temperature and/or direct contact with combustion gases, such as smoking, toasting, roasting or grilling may be responsible for high PAH levels in processed foodstuffs. The amount of PAHs generated during the thermal food processing depends on several parameters such as temperature, duration of the treatment, distance from the source of heating, oxygen accessibility, fat content, and type of combustible used (Visciano et al., 2006).

Ghana is significantly endowed with valuable fish stocks and a strong tradition and culture of fishing comparable to other West African nations (Afoakwa et al., 2018). Indeed, the country produces on the average 440,000 tons of fish from its waters each year. Ghana is both an exporter and importer of fish (FAO, 2006). Over the years, Ghana has exported large volumes of fish to international markets and as many as 2.2 million people in Ghana are dependent on the sector for their livelihoods, including some 135,000 fisher men and women in the marine sector alone (Berchie, 2012). Revenues from the fisheries sector accounts for about US\$1 billion per year, contributing at least 4.5 percent to Ghana's GDP (FAO, 2006). As a matter of fact, the country is a regular supplier of smoked fish to neighboring countries such as Togo, Benin, Cote d'Ivoire and Nigeria. It is noteworthy that fish supplies naturally augment food availability. Fish availability ensures food security and good nutritional outcomes particularly for poor and rural populations.

Fisheries products supply 60 percent of the animal protein consumed in the country and per capita fish consumption is 27 kg per year – more than double the world average. Fresh, but more often smoked, dried, or even as powder, fish is a critical source of dietary protein and micronutrients such as iron, iodine, zinc, calcium, vitamin A and vitamin B for many communities in rural areas particularly in the poorest regions of Ghana. Therefore, fish is an important source of animal protein in Ghana with 75 % of the total annual domestic production of fish consumed locally (MOFA, 2017). The traditional methods of fish processing and preservation comprise smoking, drying, frying, salting and fermenting and in some circumstances a combination of these methods. It had been reported that 95% of fish processed in Ghana is smoked, 3 % salted while the remaining 2 % is dried, fried or fermented (SMFP, 2015).

Unfortunately, the fish production in the country is confronted with a number of challenges. It is worthy to note that fish is exposed to microbial and chemical contamination right from the catch, through the processing, storage and final display at various local markets. Smoking preserves and enhances flavor; however, as the fish gets in contact with the smoke from the firewood, polycyclic aromatic hydrocarbons (PAHs) are formed which are released onto the fish product. PAHs are a class of high lipophilic compounds that comprise of chemical compounds known to be potent carcinogens (Simko, 2002). Structurally, they consist of one or more aromatic ring and their structure is known to influence their toxicity.

PAHs formation in smoked foods depend on several variables in the smoking process, including type of smoke generator/stove, type of fuel used, combustion temperature, and degree of smoking (Garcia and Simal, 2005). Other factors include; fat content of fish species and cooking time. The smoking industry in Ghana is largely unregulated with various oven types, hygienic issues and wood fuels used for smoking; as such the quality of smoked fish varies from place to place. Fish smoked in Ghana is sold in-country with some units exported

to other regional markets. Accessing the EU market has however proven quite difficult as the Union demands adherence to stringent regulations and standards regarding the sourcing, handling and processing of smoked fish (especially issues concerning Polycyclic Aromatic Hydrocarbons in smoked fish).

Research focused on quantifying the various PAH levels in different fish species of both smoked and fresh fish on the Ghanaian market should be able to provide scientific information and risk exposure to the ordinary consumer. Further analysis into microbiological profiles of fish both smoked and fresh was carried out by sampling fish from the popular sales points and markets in Ghana to be able to determine their contamination levels. Based on these results and existing information (FAO), SNV in consultation with project partners and national stakeholders aim to develop smoked fish production protocols to reduce contamination levels in smoked fish. Also, smoked, salted and fresh fish samples were found to carry various loads of microorganisms including bacteria, molds and yeasts. This study reiterates the need to intensify education on hygienic and best processing practices of fish.

Globally, the consumption of fish and fishery products has generally increased in recent decades (Wim et al., 2007) due to a shift from animal protein to fish protein which has less cholesterol levels (Shrivastava et al., 2011). However, the growing demand for aquatic products in both developing and developed nations has compelled the need to maintain the present per capita consumption of aquatic products in the future. The quality and safety of fish and fishery products as a major protein source has therefore become a major issue around the world (Huss et al., 2003).

Petran (2012) carried out a food safety analysis and established that globally, food and water borne illnesses have resulted in 2.2 million deaths out of the total 1 billion reported cases in 2012. Finfish was the second product implicated for food borne illnesses in the United States while fish and fishery products ranked fifth in the EU countries. Salmonella infestation was the main cause of all FDA's food recalls (recalls due to biological/pathogen infestation) in 2010. Salmonella infestations have been traced to foods consumed outside the home (in restaurants, pubs, hotels and bars- 44 and 32 in 2010 for USA and Europe respectively) and the source of microbes linked to the infestation of handlers at these eateries (Petran, 2012).

Primarily, fish toxicological and ecological studies have prompted interest in the determination of toxic metals (Shrivastava et al., 2011). International organizations such as the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) are working in various ways using varied regulatory mechanisms such as the Hazard Analysis and Critical Control Point (HACCP), Codex Alimentarius and the ISO 9000 series to control the infection and transmission of diseases associated with food products. Hazards associated with food may be biological, chemical or physical. Pathogens and heavy metal contamination which cause long term effects and allergens are common sources of food borne illnesses.

Microbial contamination is caused by microorganisms known as bacteria (Adams and Moss, 2000). Bacteria are found almost everywhere in the environment including soil, water, plants, animals and humans (Baird-Parker, 2000). The main carriers of bacteria are the foods that we consume which are rarely sterile (Adams and Moss, 2000). Food carries microbial associations whose composition depends on the organisms' access to food, their ability to grow and the interaction in the foods as time progresses (Adams and Moss, 2000).

Moreover, the exact origin of bacterial contamination in food depends on the natural microflora of the raw material and those organisms introduced in the course of harvesting, slaughter, processing, storage conditions and the distribution of food (Adams and Moss, 2000). Microbial contamination cause infections that are responsible for various food borne

illnesses and diseases (Collins et al., 1989). In uncontrolled conditions, these infections could cause major foodborne disease outbreaks in a community or population. Espejo-Hermes (1998) reported that infections caused by these microbes are more pronounced in developing countries, where there are improper practices in handling, storage, processing and distribution of food and food products.

Contamination of seafood by these pathogens is also a contributing factor towards human morbidity (Ripabelli et al., 2004). These bacteria gain access to the human body by direct contact with infected fish during improper handling, or being in contact with other constituents of the fish environment (Acha and Szyfres, 2003). Individuals can be infected by fish bites and fish fins which contribute to microbial contaminants gaining entry (Novotny et al., 2004).

The Ghanaian fishing industry has a long history. It has been an important source of livelihood for the people along the coast (Mensah et al., 2002). The sector is an important player in the country's economy. It is estimated to have contributed about 3.9% of the nation's Gross Domestic Product (GDP) and 11% of the Agricultural GDP in 2008 (Bank of Ghana, 2008). These GDP and AGDP figures stood at 3% and 5% respectively in 1997 (Sarpong, 2008), indicating the significant increases in the contributions of the sector to poverty reduction and provision of sustainable livelihoods over the years. The fishery sector started with very crude and inefficient harvest technology, mostly the use of traditional dugout canoes.

It has been estimated that more than 80 % of fish landed along the coast of Ghana is traditionally processed (Adu-Gyamfi, 2006). Traditional fish processing is thus an important economic activity in Ghana. It serves as a source of income to many and also provides the main form in which fish is consumed. According to Sefa-Dedeh (1993), traditional fish processing is often characterized by all or most of the following: low capital cost, time consuming, labor intensive, simple and small scale operations, poor quality control, Home based, and Unhygienic processing conditions.

The various methods of traditional fish processing in Ghana are smoking, salting, drying, fermentation, and frying (Neequaye-Tetteh et al., 2002). Among these, smoking is the commonest with more than 60 % of the country's fish landings preserved by smoking (Adu-Gyamfi, 2006). Traditionally, smoked fish has also been the most patronized of all traditionally processed fish in Ghana (Adu-Gyamfi, 2006). UNDP, (2002), has also documented high level of smoked fish processing and consumption for other West African countries.

The Ghana Standards Authority has defined smoked fish as fish which has been exposed to smoke with the intention of deferring spoilage. Traditional fish smoking preserves fish through the combined effects of the following:

**Cooking:** at high temperatures, the fish are cooked, thereby denaturing enzymes which could cause deterioration, and eliminating vegetative microorganisms that could cause spoilage.

**Drying:** heat from the burning wood contributes to the drying of the fish.

**Preservation value of the smoke:** compounds such as methanol and phenols in the smoke have bactericidal properties (Holley and Patel, 2005).

Smoked fish are placed into two categories based on the processing temperature at which they are produced. These are cold-smoked and hot-smoked fish (UNDP, 2002). In cold-smoking, the internal temperature of the fish usually does not exceed 35o C. Generally, a range of 30-40o C for 30-60 minutes is typical (Cofie, 2003). It is common in technologically

advanced societies. Cold-smoked fish are neither well dried nor cooked due to the low temperatures employed. Hence, they have high moisture contents and short shelf-life, usually 3 days (Cofie, 2003). They mostly require cooking before consumption. In hot-smoking, the processing temperature is typically greater than 90°C. The internal temperature of fish typically exceeds 60 °C.

The products have relatively low moisture content and thus have longer shelf life. Hot-smoked fish are cooked and can therefore be consumed without further heat treatment (Bannerman and Cowx, 2002). Hot-smoking is the method employed in traditional fish smoking in Ghana, and in many developing countries (UNDP, 2002). There are two forms of hot-smoking, namely wet hot-smoking and dry hot-smoking. They differ in their duration and the final moisture content of the products. Wet hot-smoking normally takes 1-2 hours and yields a product with moisture contents of 40-55 %, while dry hot-smoking usually takes 10-18 hours and yields products with low moisture contents 10-15 % (UNDP, 2002).

Supporting these findings, fish and fishery products have been the most preferred and cheapest source of animal protein in Ghana (Adu-Gyamfi, 2006). Approximately 75 % of total annual fish catch in Ghana is locally consumed (BOG, 2008). The high consumption rate is largely due to its high availability and low price of fish compared to other sources of animal protein. Given that about 80 % of fish catch in Ghana is traditionally processed (smoked, salted, fried, or dried), it can be said that a greater amount of the 75 % of total annual fish landings consumed in the country is traditionally processed.

By extension, it can be said that traditionally processed fish possibly constitutes a greater percentage of the 60 % animal protein provided by fish in Ghanaian diets, and that a greater percentage of the predicted 22.4 % household expenditure on fish is made of the traditionally processed fish. It is therefore reasonable to suggest that Ghana is heavy consumer of traditionally processed fish. These products are mostly obtained from informal markets in both urban and rural areas. These informal markets are indispensable component of the fishery sector in Ghana. Ovens are built in front of homes in compound houses. Areas used for drying, processing areas, materials and activities are not well separated from other households thus enhancing the possibility of cross contamination.

Fish is a rich source of protein, essential acids like omega 3 fatty acids, proteins, vitamins and minerals with a flesh pH of about neutral (pH~7). These characteristics make it an ideal suitable living and proliferation medium for bacteria and harmful pathogens from contaminated waters and unsanitary landing beaches. Consumption of such fish may be injurious to human health by causing infections and intoxication. Fish contamination comes from a variety of sources. Freshly caught fish from unpolluted water is largely sterile. The skin, viscera and gills get contaminated to varying degrees depending on the environment in which they are caught. Additional contamination of fish may occur on canoes or on land.

Also depending on the level of application of Good Manufacturing Practices, contamination may take place on board through: eviscerating, rinsing and storage in ice. On land, contamination may be through the following operations: unloading, sorting, filleting, gutting, portioning, packing and transporting. Fish in uncontaminated water may contain 102 CFU/g and 103 CFU/g on skin and viscera, respectively (Adams and Moss, 2003). In polluted tropical and sub-tropical waters, contamination of bacteria may increase from 107 to 109 CFU/g in the skin and viscera respective. Shellfish in cold water contains 105 bacteria/gram and that from warm water contains 105 to 106 bacteria/gram. In mollusks such as oysters and mussels 104 to 106 CFU/g bacteria/gram may be present (Adams and Moss, 2003). Fresh fish from warm tropical waters may be contaminated with Gram positive bacteria such as *Corynebacterium*, *Bacillus*, and *Micrococcus*. When stored in ice however, over 90 %

*Pseudomonas* spp. and *Shewanella* spp. are present. Fresh fish caught in polluted areas or fish that are not properly treated on land or on board, can be contaminated with pathogens such as: *Salmonella*, *Enterococci*, *Staphylococcus aureus*, *Clostridium botulinum* type E. In living fish, two pathogens may survive, namely *Clostridium botulinum* type E and *Vibrio parahaemolyticus* (in warm water) (Colakoglu et al., 2006).

Conventionally, three major means: (a) education and training, (b) inspection of facilities and operations, and (c) microbiological testing have been used by Food Safety Inspectors and Food Business Operators to control microorganisms in food. These programs have been directed toward developing an understanding of the causes and consequences of microbial contamination and to evaluate facilities, operations and adherence to good best practices. Although these are critical parts in any food safety programme, they have certain limitations and weaknesses. Enumeration of microbial counts in food is often used in the retrospective assessment of microbiological quality or to assess the presumptive “safety” of foods. This procedure requires that food is sampled, microbiological analyses are performed and the results assessed by comparing with already established microbiological specification (FAO/CDR, 2013). As far as inspection of facilities and operations is concerned, this is often carried out with reference to various guidelines such as best hygienic practices and food control laws.

These measures mostly do not give the significance of the various requirements, which are often stated in vague terms such as “satisfactory”, “adequate”, “acceptable”, “suitable”, “if necessary”. This lack of specificity leaves the interpretation to the Food Hygiene Officer who uses his or her discretion in most cases. The Inspector may place little emphasis on very important matters and thus increase costs without necessarily reducing food safety hazards. Microbial examinations are carried out to detect the presence of pathogenic bacteria (*V. Parahaemolyticus*, *E. coli*) or for microorganisms which gives indications of faecal Contamination or other types of general contamination or poor hygienic practices (coliform bacteria, faecal *Streptococci* (FAO/CDR, 2013). Also, it should be emphasized again that a negative test for specific pathogens in a food sample is not an assurance that the whole lot is free of these pathogens (FAO/CDR, 2013).

Thus only a very limited degree of safety can be obtained by microbiological analyses. The other tests come with a number of limitations. Total Viable Count (TVC) or Aerobic Plate Count (APC) is defined as the number of microorganisms (CFU/g) in a food product obtained under optimal conditions of culturing. Thus, the TVC is not a guarantee of the “total” bacterial population, but only a measure of the fraction of the microflora able to produce colonies in the medium used under the conditions of incubation. Thus it is well known that the Conditions during incubation influence greatly the number of colonies developing from the same sample. As an example, the TVC may vary by a factor 10–100 when iced fish is sampled and Plates are incubated at 20 °C and 37 °C respectively.

Furthermore, the TVC does not differentiate between different types of bacteria and similar levels of TVC may therefore be found although the biochemical activity of the bacteria may vary widely in the food. Also, high counts as a result of microbial growth are much more likely to cause defects in foods. TVC is therefore of no value in assessing the present state of organoleptic characteristics. It is of very doubtful value in the examination of frozen fish products (FAO/CDR, 2013). An unknown and uncontrolled kill or damage of the bacteria may have taken place during freezing and cold storage. A very low “total” count may therefore lead to false conclusions about the hygienic quality of the product. Tests for TVC may be useful for measuring the conditions of the raw material, effectiveness of procedures (i.e. heat treatment) and hygiene conditions during processing, sanitary conditions of



equipment and utensils. However, to be useful and for correct interpretation of results a thorough knowledge of handling and processing conditions prior to sampling is essential. Current studies have shown that *E. coli* and faecal coliform bacteria can be found in unpolluted warm tropical waters and that *E. coli* can survive indefinitely in this environment (Hamed et al., 2013).

These findings also revealed that there was no correlation between presence or absence of faecal coliforms, total coliforms and virus (Hamed et al., 2013). Thus, in the tropics *E. coli* or faecal coliforms are not reliable of recent biological contamination or sewage effluent discharge into aquatic bodies. This point should be taken into consideration when microbiological criteria are applied to fish and fishery products from tropical countries. A microbiological criterion is a standard against which comparison and assessment of research data may be made. The standard may have either obligatory or optional status. A microbiological standard is a microbiological criterion that is part of a law or ordinance and is an obligatory criterion. A microbiological guideline is a standard used to assess microbiological conditions during the production chain (processing, distribution and marketing of foods) hence it is mostly an advisory criterion. A microbiological specification is used in purchase agreements between buyer and supplier. Microbiological criteria may be useful in evaluating the safety and shelf-life of foods, the adherence to established Good Operational Best Practices and the correctness of food for a specific purpose.

### 3. METHODOLOGY

#### Data collection for microbiological studies

Smoked *Sardinella aurita* and *Sardinella maderensis* were collected from active fish markets at the coastal regions of Ghana (Figure 1). In all, a total of four fish markets (i.e. one in each coastal region) was visited for the purchasing of smoked fishes (*Sardinella aurita* and *Sardinella maderensis*). Furthermore, fresh *Sardinella aurita* and *Sardinella maderensis* were purchased from retail shops and landing beaches in Tema and Elmina located in Greater Accra and Central regions respectively (Figure 1).

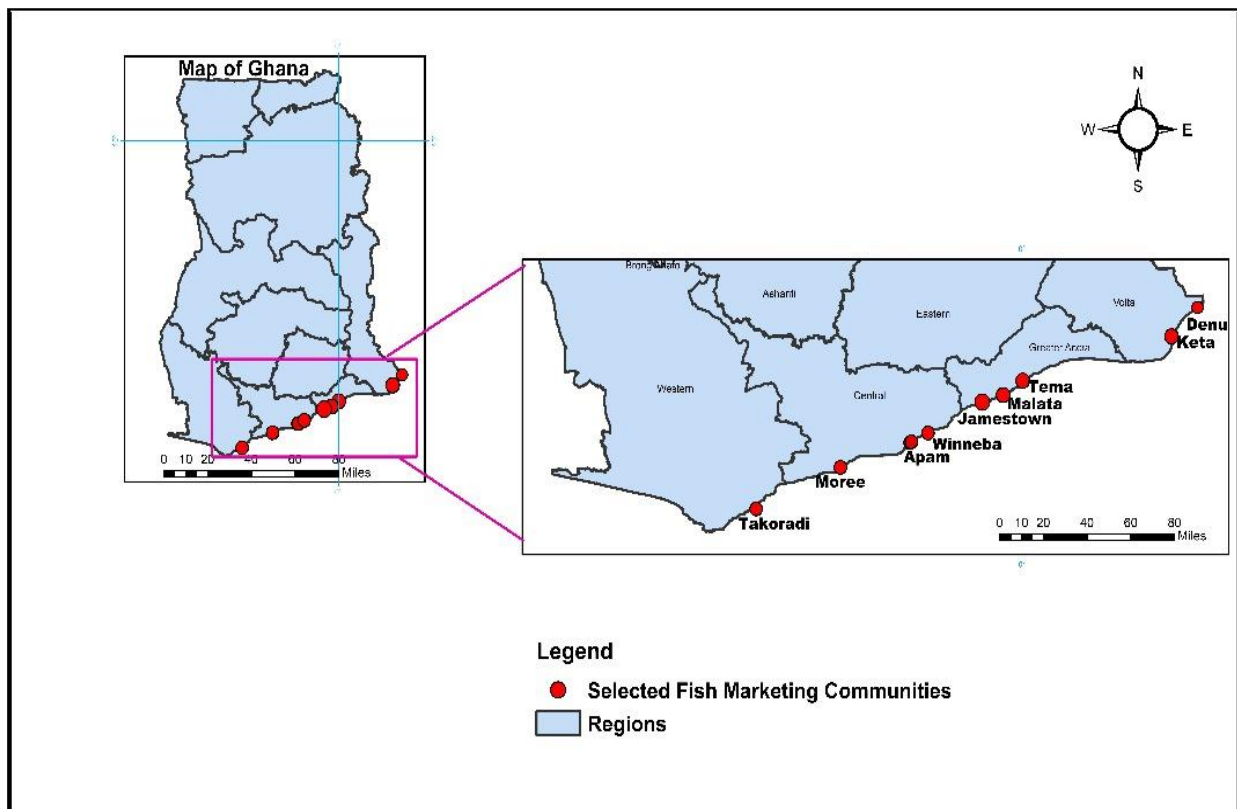


Figure 1. Map showing the study areas

#### Data collection for PAH studies

Smoked *Sardinella aurita* was collected from fish smokers who used barrel oven, Chorkor oven and Ahotor oven in smoking. Parameters including the type of wood, smoking time and temperature were collected from these fish smokers.

#### Sample preparation

Both fresh and smoked *Sardinella aurita* and *Sardinella maderensis* were wrapped in aluminium foil and transported on ice to the Microbiological Laboratory at the CSIR-Food Research Institute, Ghana for microbiological analysis.

For PAH, samples obtained were homogenized at the Microbiological Laboratory at the CSIR-Food Research Institute, Ghana and transported to Institute of Marine Research, Norway for PAH analysis.

## Risk assessment of PAH in smoked fishes

Risk assessment of PAHs in smoked *Sardinella aurita* were determined by comparing the total toxicity equivalent concentration (TTEC) value to the screening value of PAH in the samples (Cheung et al., 2007; Nyarko et al., 2011). The TTEC value was obtained by summing the products (BaP<sub>eq</sub>) of all the PAH congeners by their corresponding toxicity equivalency factor (TEF) as expressed in equation 1.

$$\text{TTEC} = \Sigma(\text{Concentrations of PAH congeners} \times \text{TEF}) \dots \dots \dots (1)$$

Where, TTEC is the total toxicity equivalent concentration and TEF is the toxic equivalency factors (Nyarko et al., 2011). If the TTEC was greater than the screening value (SV) then risk was implicated but if less than SV then, risk was significantly negligible.

The screening value were computed and expressed in excel as equation 2.

$$\text{SV} = (\text{RL}/\text{SF} * \text{BW})/\text{CR} \dots \dots \dots (2)$$

Where, SV is the screening value (µg/g); RL is the maximum acceptable risk level, (dimensionless), SF is the oral slope factor (µg/g day), BW is the body weight (g) and CR is the consumption rate (g/day) (Nyarko et al., 2011).

## 4. RESULTS

### Microbiological profiling

#### Smoked Sardines

Tables 1 and 2 show the microbiological quality of smoked *Sardinella aurita* and *Sardinella maderensis* species from the sampling stations. From Table 1, *E. coli* and *Staphylococcus aureus* were not detected from samples obtained from markets in all the samples. *Clostridium perfringens* were not detected in smoked samples from Winneba, Malata and Takoradi. However, samples from Keta market recorded 100 cfu/g of *Clostridium perfringens* (Table 1). Though samples from Winneba and Takoradi did not record any *Enterococcus* organisms, smoked fish samples from Keta and Malata recorded 3900 cfu/g and 140 cfu/g, respectively (Table 1).

**Table 1. Microbiological profile of smoked *Sardinella aurita* (Round sardine)**

| Location | <i>E.coli</i> | <i>Enterococcus</i> | <i>Staphylococcus aureus</i> | <i>Clostridium perfringens</i> |
|----------|---------------|---------------------|------------------------------|--------------------------------|
| Keta     | Not Detected  | 3900                | Not Detected                 | 100                            |
| Winneba  | Not Detected  | Not Detected        | Not Detected                 | Not Detected                   |
| Malata   | Not Detected  | 140                 | Not Detected                 | Not Detected                   |
| Takoradi | Not Detected  | Not Detected        | Not Detected                 | Not Detected                   |

*E. coli* and *Staphylococcus aureus* were not detected from smoked *Sardinella maderensis* (Flat sardine) samples obtained from markets in all the samples (Table 2). *Clostridium perfringens* were not detected in smoked *Sardinella maderensis* samples from Winneba, Malata and Takoradi. However, *Sardinella maderensis* samples from Keta market recorded 20 cfu/g of *Clostridium perfringens* (Table 2). Though smoked *Sardinella maderensis* samples from Takoradi did not record any *Enterococcus* organisms, smoked *Sardinella maderensis* samples from Winneba, Keta and Malata recorded 360 cfu/g, 1800 cfu/g and 110 cfu/g, respectively (Table 2).

**Table 2. Microbiological profile of smoked *Sardinella maderensis* (Flat sardine)**

| Location | <i>E. coli</i> | <i>Enterococcus</i> | <i>Staphylococcus aureus</i> | <i>Clostridium perfringens</i> |
|----------|----------------|---------------------|------------------------------|--------------------------------|
| Keta     | Not Detected   | 1800                | Not Detected                 | 20                             |
| Winneba  | Not Detected   | 360                 | Not Detected                 | Not Detected                   |
| Malata   | Not Detected   | 110                 | Not Detected                 | Not Detected                   |
| Takoradi | Not Detected   | Not Detected        | Not Detected                 | Not Detected                   |

#### Fresh sardine samples

*Clostridium perfringens* and *Staphylococcus aureus* were not detected from fresh *Sardinella maderensis* (Flat sardine) samples obtained from markets in all the samples (Table 2). However, fresh *Sardinella maderensis* samples from Tema market recorded 80 cfu/g and 40 cfu/g of *E. coli* and *Enterococcus*, respectively (Table 3).

**Table 3. Microbiological profile of fresh *Sardinella maderensis* (Flat sardine)**

| Location | <i>E. coli</i> | <i>Enterococcus</i> | <i>Staphylococcus aureus</i> | <i>Clostridium perfringens</i> |
|----------|----------------|---------------------|------------------------------|--------------------------------|
| Madina   | Not detected   | Not detected        | Not Detected                 | Not Detected                   |
| Tema     | 80             | 40                  | Not Detected                 | Not Detected                   |
| Elmina   | Not detected   | Not detected        | Not Detected                 | Not Detected                   |

*Clostridium perfringens* and *Staphylococcus aureus* were not detected from fresh *Sardinella aurita* (Round sardine) samples obtained from all the markets (Table 2). However, fresh *Sardinella aurita* samples from Tema market recorded 70 cfu/g and 60 cfu/g of *E. coli* and *Enterococcus*, respectively (Table 4).

**Table 4. Microbiological profile of fresh *Sardinella aurita* (Round sardine)**

| Location | <i>E. coli</i> | <i>Enterococcus</i> | <i>Staphylococcus aureus</i> | <i>Clostridium perfringens</i> |
|----------|----------------|---------------------|------------------------------|--------------------------------|
| Madina   | Not detected   | Not detected        | Not Detected                 | Not Detected                   |
| Tema     | 70             | 60                  | Not Detected                 | Not Detected                   |
| Elmina   | Not detected   | Not detected        | Not Detected                 | Not Detected                   |

## Polycyclic aromatic hydrocarbon

### **Benzo (a) pyrene**

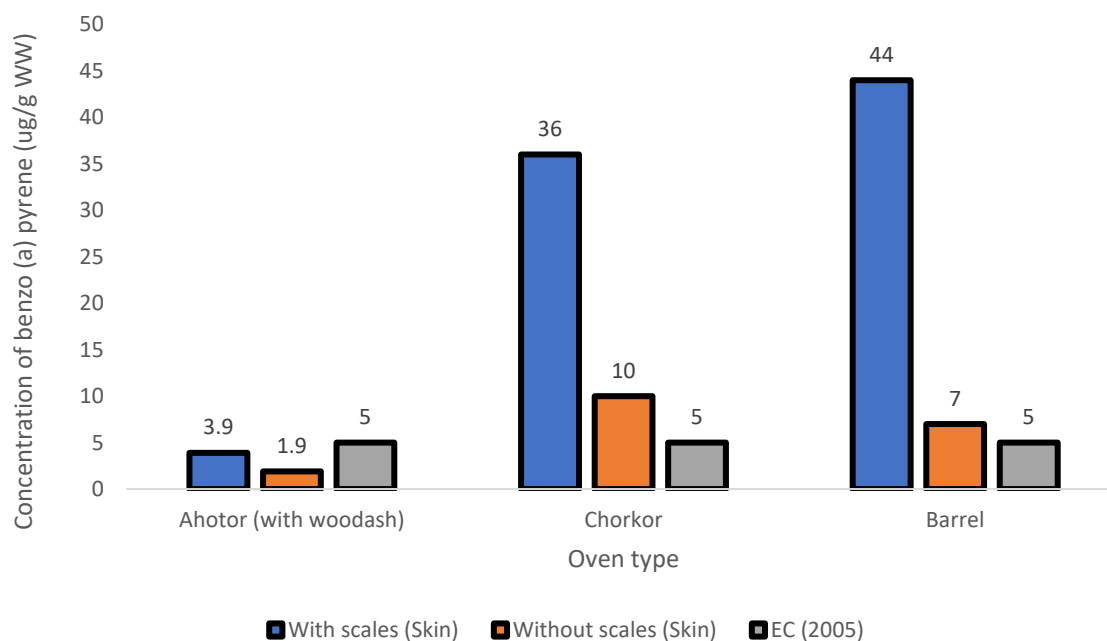
For smoked *Sardinella aurita* with the skin, Benzo (a) pyrene values ranged from 3.9 ug/g WW to 44 ug/g WW (Figure 2). Smoked samples from the Ahotor oven (with wood ash) recorded the lowest Benzo (a) pyrene value of 3.9 ug/g WW followed by smoked samples from Chorkor oven (36 ug/g WW) with Barrel oven samples recording the highest value of Benzo (a) pyrene (44 ug/g WW).

For smoked *Sardinella aurita* without the skin, Benzo (a) pyrene values ranged from 1.9 ug/g WW to 10 ug/g WW (Figure 2). Smoked samples from the Chorkor oven recorded the highest Benzo (a) pyrene value of 10 ug/g WW with samples from Ahotor oven (with wood ash) recording the least value of 1.9 ug/g WW (Figure 2).

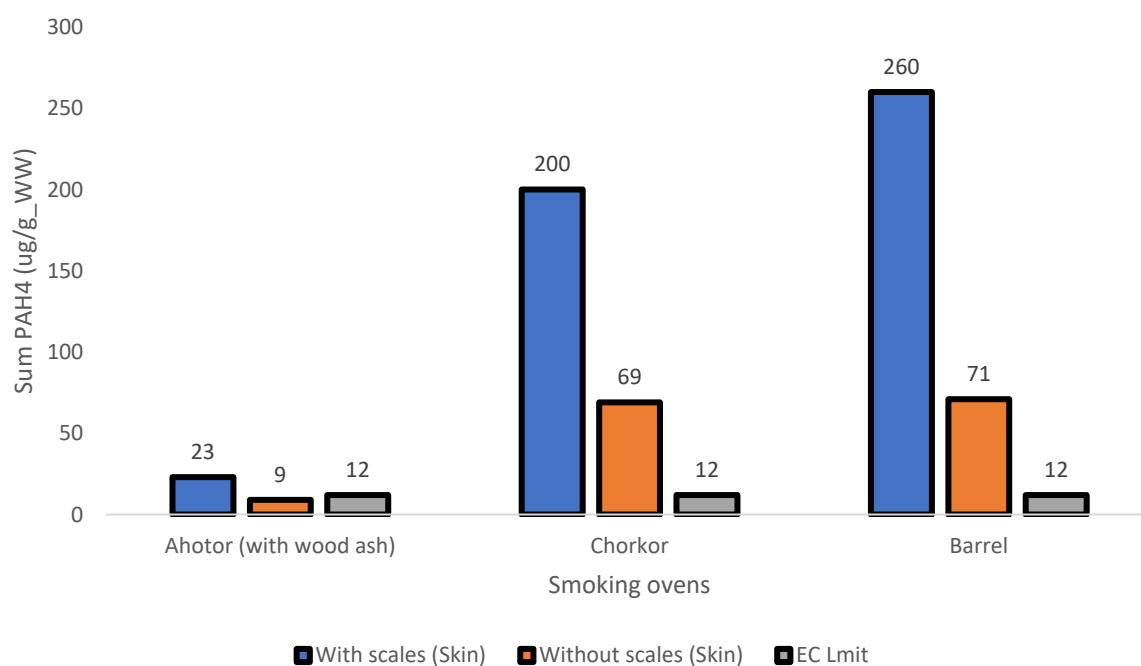
### **Sum of PAH<sub>4</sub>**

For smoked *Sardinella aurita* with the skin, sum of PAH<sub>4</sub> values ranged from 23 ug/g WW to 260 ug/g WW (Figure 3). Smoked samples from the Barrel oven recorded the highest sum of PAH<sub>4</sub> value of 260 ug/g WW followed by smoked samples from Chorkor oven (200 ug/g WW) with Ahotor oven (with wood ash) samples recording the least value of sum of PAH<sub>4</sub> (23 ug/g WW).

For smoked *Sardinella aurita* without the skin, sum of PAH<sub>4</sub> values ranged from 9 ug/g WW to 71 ug/g WW (Figure 3). Smoked samples from the Ahotor oven (with wood ash) recorded the lowest sum of PAH<sub>4</sub> value of 9 ug/g WW followed by smoked samples from Chorkor oven (69 ug/g WW) with Barrel oven sample recording the highest value of 71 ug/g WW (Figure 3).



**Figure 2. Benzo (a) pyrene levels in smoked *Sardinella aurita* (Round sardinella) from different ovens**



**Figure 3. Sum of PAH4 levels in smoked *Sardinella aurita* (Round sardinella) from different ovens**

### Cancer risk assessment

Table 5 shows the Toxicity Equivalent Factors (TEF) for the PAH congeners used in the estimation of the Total Toxicity Equivalent concentration for the various smoked fish samples from the assessed fish smoking ovens.

**Table 5. TEFs of some PAHs congeners**

| PAH congeners         | Concentration (mg/g) <sup>1</sup> | TEF factors |
|-----------------------|-----------------------------------|-------------|
| Benz(a)anthracene     | 75                                | 0.1         |
| Benzo(a)pyrene        | 44                                | 1           |
| Benzo(b) fluoranthene | 47                                | 0.1         |
| Chrysene              | 93                                | 0.01        |

The Total Toxicity Equivalent Concentration (TTEC) of smoked *Sardinella aurita* samples from the various ovens are represented in Figure 4. From Figure 4, smoked fish samples with the skin as compared to fish samples without the skin recorded the highest amount of TTEC (i.e. 10.6, 46.85 and 57.1 as against 1.75, 10.6 and 13.6 for Ahotor (with wood ash), Chorkor and Barrel ovens respectively).

Regarding smoked fish samples with the skin, samples from the Barrel oven recorded the highest value (57.1) followed by smoked samples from the Chorkor oven (46.85) with Ahotor oven recording the least (26.6). Similar observation was recorded for smoked fish samples without the skin (Figure 4). For smoked fish samples with the skin, samples from the Barrel oven recorded the highest value (57.1) followed by smoked samples from the Chorkor oven (46.85) with Ahotor oven (without wood ash) recording the 26.6 with samples from Ahotor oven (with wood ash) recording the least (10.6) as shown in Figure 4. From Equation (2) in Chapter 2, the Screening value (SV)<sup>2</sup> was estimated as 0.0014 µg/g.

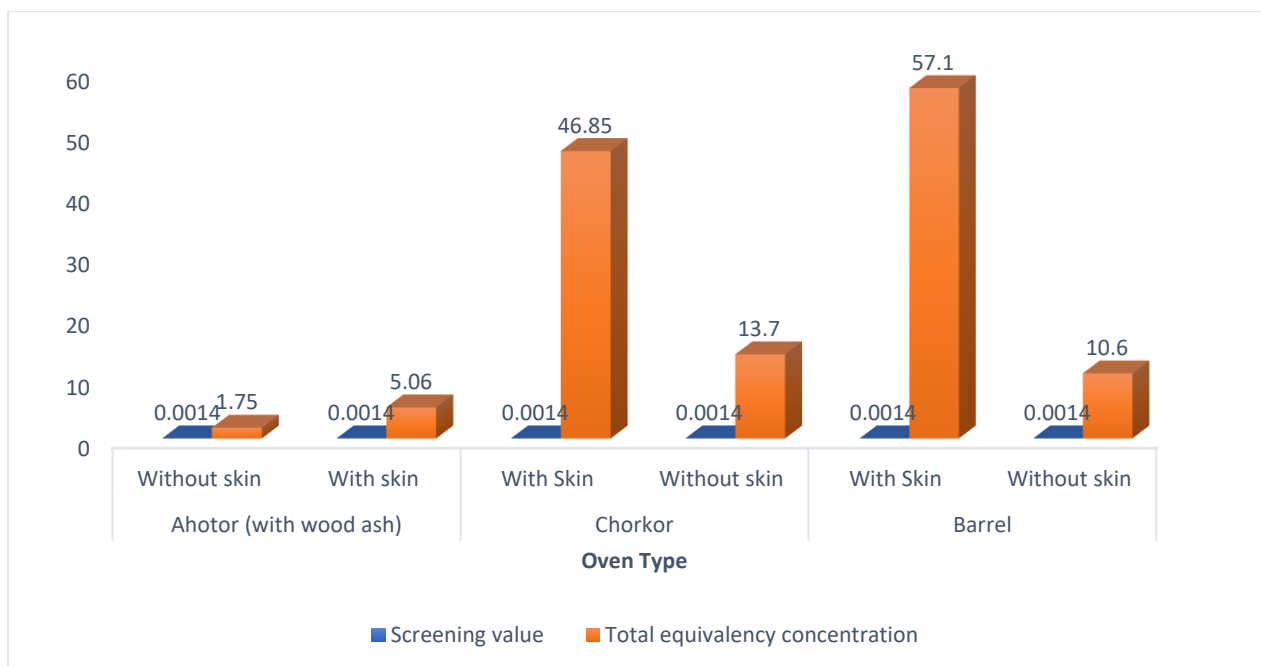
The carcinogenic and mutagenic toxicity in relation to BaP were calculated for the carcinogenic and mutagenic risk associated with ingestion of smoked fish (both with the skin and without the skin). TEQ<sub>BaP</sub> are largely associated with cancerous diseases while MEQ<sub>BaP</sub> is linked to non-cancerous disorders including low intelligent quotient, impotency, birth defects and pulmonary disorders.

For carcinogenic risk of smoked fish with the skin, the corresponding EQ<sub>BaP</sub> daily dose for an adult of 70 years' ingestion of smoked fish (with the skin) from the assessed smoking ovens were calculated as  $5.49 \times 10^{-5}$ ,  $3.09 \times 10^{-5}$  and  $5.88 \times 10^{-6}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively (Table 6). Corresponding carcinogenic risk for an adult of 70 years' ingestion of smoked fish (with the skin) from the assessed smoking ovens was calculated as follows  $3.2 \times 10^{-4}$ ,  $1.63 \times 10^{-4}$  and  $3.47 \times 10^{-5}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively (Table 6).

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<sup>1</sup> mg/g = µg/g.

<sup>2</sup> RL = 0.00001  
SF = 7.3 µg/g day  
BW = 70000 g  
CR = 68.5 g/day



**Figure 4. Total Toxicity Equivalent concentration of smoked samples from the Barrel, Chorkor and Ahotor ovens**

**Table 6. Risk assessment based on carcinogenic equivalency, average daily dose and risk for smoked fish samples (with skin) using the traditional and modern ovens**

| Carcinogenic equivalency    | Chorkor oven | Barrel oven | Ahotor oven (wood ash) |
|-----------------------------|--------------|-------------|------------------------|
| Benz(a)anthracene           | 6.6          | 5.8         | 0.7                    |
| Benzo(a)pyrene              | 36           | 18          | 3.9                    |
| Benzo(b)fluoranthene        | 3.6          | 2.2         | 0.34                   |
| Chrysene                    | 0.065        | 0.062       | 0.009                  |
| BaP-TEQ                     | 46.27        | 26.06       | 4.95                   |
| BaPEQ daily dose            | 5.49E-05     | 3.09E-05    | 5.88E-06               |
| Life-time Carcinogenic risk | 3.20E-04     | 1.63E-04    | 3.47E-05               |

For mutagenic risk of smoked fish with the skin, the corresponding  $EQ_{BaP}$  daily dose for an adult of 70 years' ingestion of smoked fish (with the skin) from the assessed smoking ovens were calculated as  $6.10 \times 10^{-5}$ ,  $3.48 \times 10^{-5}$  and  $6.50 \times 10^{-6}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively (Table 7). Corresponding mutagenic risk for an adult of 70 years' ingestion of smoked fish (with the skin) from the assessed smoking ovens were calculated as follows  $3.2 \times 10^{-4}$ ,  $1.65 \times 10^{-4}$  and  $3.50 \times 10^{-5}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively.



**Table 7. Risk assessment based on mutagenic equivalency, average daily dose and risk for smoked fish samples (with skin) using the traditional and modern ovens**

| Mutagenic equivalency    | Chorkor oven | Barrel oven | Ahotor oven (wood ash) |
|--------------------------|--------------|-------------|------------------------|
| Benz(a)anthracene        | 5.4          | 4.8         | 0.6                    |
| Benzo(a)pyrene           | 36           | 18          | 3.9                    |
| Benzo(b)fluoranthene     | 9            | 5.5         | 0.9                    |
| Chrysene                 | 1.1          | 1.1         | 0.2                    |
| BaP-MEQ                  | 51.5         | 29.3        | 5.5                    |
| BaPEQ daily dose         | 6.10E-05     | 3.48E-05    | 6.50E-06               |
| Life-time Mutagenic risk | 3.20E-04     | 1.65E-04    | 3.50E-05               |

For carcinogenic risk of smoked fish without the skin, the corresponding EQ<sub>BaP</sub> daily dose for an adult of 70 years' ingestion of smoked fish (without skin) from the assessed smoking ovens were calculated as  $1.61 \times 10^{-5}$ ,  $1.20 \times 10^{-5}$  and  $2.00 \times 10^{-6}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively (Table 8). Corresponding carcinogenic risk for an adult of 70 years' ingestion of smoked fish (without skin) from the assessed smoking ovens were calculated as follows  $8.97 \times 10^{-5}$ ,  $6.34 \times 10^{-5}$  and  $1.16 \times 10^{-5}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively (Table 8).

**Table 8. Risk assessment based on carcinogenic equivalency, average daily dose and risk for smoked fish samples (without skin) using the traditional and modern ovens**

| Carcinogenic equivalency    | Chorkor oven | Barrel oven | Ahotor oven (wood ash) |
|-----------------------------|--------------|-------------|------------------------|
| Benz(a)anthracene           | 2.4          | 5.8         | 0.7                    |
| Benzo(a)pyrene              | 10           | 18          | 3.9                    |
| Benzo(b)fluoranthene        | 1.1          | 2.2         | 0.34                   |
| Chrysene                    | 0.024        | 0.062       | 0.009                  |
| BaP-TEQ                     | 13.52        | 26.06       | 4.95                   |
| BaPEQ daily dose            | 1.61E-05     | 1.20E-05    | 2.00E-06               |
| Life-time Carcinogenic risk | 8.97E-05     | 6.34E-05    | 1.16E-05               |

For mutagenic risk of smoked fish without the skin, the corresponding EQ<sub>BaP</sub> daily dose for an adult of 70 years' ingestion of smoked fish (without skin) from the assessed smoking ovens were calculated as  $1.80 \times 10^{-5}$ ,  $1.34 \times 10^{-5}$  and  $2.00 \times 10^{-6}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively (Table 9). Corresponding mutagenic risk for an adult of 70 years' ingestion of smoked fish (without skin) from the assessed smoking ovens were calculated as follows  $9.07 \times 10^{-5}$ ,  $6.40 \times 10^{-5}$  and  $1.16 \times 10^{-5}$  for fish samples from Chorkor oven, Barrel oven and Ahotor oven respectively (Table 9).

**Table 9. Risk assessment based on mutagenic equivalency, average daily dose and risk for smoked fish samples (without skin) using the traditional and modern ovens**

| Mutagenic equivalency    | Chorkor oven | Barrel oven | Ahotor oven (wood ash) |
|--------------------------|--------------|-------------|------------------------|
| Benz(a)anthracene        | 1.97         | 1.89        | 0.28                   |
| Benzo(a)pyrene           | 10           | 7           | 1.3                    |
| Benzo(b)fluoranthene     | 2.75         | 2           | 0.14                   |
| Chrysene                 | 0.408        | 0.41        | 0.004                  |
| BaP-TEQ                  | 15.13        | 11.29       | 1.72                   |
| BaPEQ daily dose         | 1.80E-05     | 1.34E-05    | 2.00E-06               |
| Life-time Mutagenic risk | 9.07E-05     | 6.40E-05    | 1.16E-05               |

## 5. DISCUSSION

### Microbiological studies

*Escherichia coli* present in raw foods is seen as an indicator of direct or indirect fecal contamination. Direct contamination happens through the processing of raw foods of animal origin and poor personal hygiene of food handlers. Indirect contamination of *Escherichia coli* occurs through sewage and polluted water used in processing raw animal-based foods. Therefore, the non-detection of *Escherichia coli* in the smoked samples could be due to the cleanliness of processing environment and personal hygiene among food handlers. The application of heat during smoking process and the selection of quality fish species (i.e. fish without bruises or cuts) could be other reasons accounting for such observation. However, at Tema landing beach where fresh sardine samples were purchased, the sanitation of the environment was very poor and most fish sellers were not seen practicing good personal hygiene. For samples from Madina, the absence of these spoilage toxins was as a result of the presentation treatment given, which is the samples were frozen. Freezing raw food as a means of preventing deterioration inhibits the growth of harmful food microorganisms.

*Clostridium perfringens* are anaerobic, motile rods and spore formers with spores being extremely heat resistant. As such they can withstand boiling for several hours. They are mostly present in protein foods including animal-based foods. Most animal-based protein foods become contaminated by its spores through the following sources, intestinal content of animals and humans, dust and sewage. However, from field observation, the presence of *Clostridium perfringens* in smoked samples (i.e. *Sardinella aurita* and *Sardinella maderensis*) could be due to the presence of a dumping site situated opposite to the market where the samples were obtained. Further to this, the dusty nature of markets in Keta from where samples were purchased may have facilitated the transfer of *Clostridium perfringens* to smoked fishes on sale.

The genus *Enterococcus* mainly includes species that were previously grouped as fecal streptococci and other streptococci. Species in this genus once established are often difficult to remove completely. They are associated with sewage and water especially polluted water. From the study, their presence in high numbers suggest poor sanitary conditions of the equipment used in selling the smoked fish (i.e. the wooden tray or metallic basin) as well as the environment in which selling was being done. However, at Tema landing beach where fresh sardine samples were purchased, the sanity of the environment was very proper and most fish sellers were not seen practicing good personal hygiene, thus, accounting for the presence of *Enterococcus* genus. For samples from Madina, the absence of these spoilage toxins was as a result of the presentation treatment, given that the samples were frozen. Freezing raw food as a means of preventing deterioration inhibits the growth of harmful food microorganisms.

*Staphylococcus aureus* are mostly present in raw animal-based foods that are extensively handled without regards to personal hygiene and temperature abused. Contaminated equipment used in processing raw animal-based foods facilitate the growth of *Staphylococcus aureus*. Therefore, the non-detection of *Staphylococcus aureus* and *Escherichia coli* in the smoked samples could be due to the cleanliness of processing environment, personal hygiene among food handlers. The application of heat during smoking process and the selection of quality fish species (i.e. fish without bruises or cuts) could be other reasons accounting for such observation.

## Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons are mostly produced as a result of the production of smoke. However, the concentration of PAH produced depends on several factors including the type of fuelwood used, the duration of smoking (cooking), the location of the smoke house and the destination of smoked fish products. From the study, the level of PAHs was seen to be highest in fish samples smoked with skin (scales) than in samples without the scales. Inferably, this finding suggests that fish skin absorbs high level of PAHs than the tissues (muscle), hence consumption of smoked fish with skin as in the case of *Scomber colias* (Chub mackerel) and other fish species may pose high risk to the health of consumers. Further to this, it is advisable for consumers to remove the skin of smoked fishes after smoking before consumption.

The relatively high amount of the Benzo (a) pyrene in smoked fish samples (with the skin) from the Barrel oven may be due to the higher amount of the lipid content. Silva *et al.* (2011) reported that biological membranes are made up of lipids with most organic pollutants being lipophilic in nature. Furthermore, it was observed that in open space, smoke from vehicles and other active ovens settles on the fish being smoked, thus increasing the level of Benzo (a) pyrene deposited on the skin of smoked fish. However, removal of the skin prior to analysis may have accounted for the reduction in Benzo (a) pyrene in smoked fish samples (without the skin) from the Barrel oven.



**Figure 5. Fish smoke compound at Jamestown**

The high level of Benzo (a) pyrene in smoked samples from the Chorkor smoker was as a result of the longer period in smoking. It is reported that, longer duration in smoking exposes the smoked fish samples to higher amount of PAH (i.e. Benzo (a) pyrene). Re-smoking during the next day in order to reduce the moisture content while enhancing its shelf-life may have accounted for the high amount of Benzo (a) pyrene observed from the study. The

relatively small size of the smoked fish samples indicates a larger surface area to the heat source, thus, increasing the amount of the Benzo (a) pyrene on samples (Figure 6). Again, the relatively big chamber for firewood accommodates more fuelwood which also adds up to amount of Benzo (a) pyrene on the smoked fish during pyrolysis (Figure 7).



Figure 6. Smoked fish completely exposure to the smoke from the Chorkor oven at Chorkor

The low level of Benzo (a) pyrene in smoked samples from the Ahotor smoker was largely due to the presence of the fat collector which functions as a barrier to fat and other drippings into the heat source directly. It is known that these drippings result in incomplete combustion hence producing high levels of Benzo (a) pyrene.

Furthermore, the position of the raw fish samples during smoking as a result of the fat collector may have also accounted for the reduced level of Benzo (a) pyrene. However, the jet propulsion of smoke design for its combustion chamber implies initial high amount of smoke on fish samples placed on the first tray, thus, fish on the first may be exposure to high amount of Benzo (a) pyrene than those on succeeding trays.



**Figure 7. Chorkor oven with its combustion chamber fully filled with fuelwood at Chorkor**



**Figure 8. Ahotor oven showing distance between fish tray and fat collector**

### **EC limitation on PAH (especially Benzo (a) pyrene)**

All the smoked fish samples from the various ovens investigated recorded levels of Benzo (a) pyrene higher than the EC limit of 5ug/g WW. Smoked fish samples with the skin recorded relatively higher amount of Benzo (a) pyrene than samples with the skin removed. This clearly indicates that Benzo (a) pyrene largely settles on the skin of smoked fishes.

Nonetheless, the use of wood ash in smoking fish using the Ahotor oven aided in the reduction of Benzo (a) pyrene to levels lower than the EC limit of 5 ug/g WW. Therefore, the use of wood ash in smoking fish using Ahotor oven is urgently advocated as it achieves the EU limit on PAH (especially Benzo (a) pyrene).

## **6. CONCLUSION AND RECOMMENDATIONS**

### **Conclusions**

The following conclusions are derived from the results:

Poor handling of smoked and fresh *Sardinella aurita* and *Sardinella maderensis* by fish processors as well as traders at the various fish landing sites. This was as a result of poor sanitation of the environment and non-adherence to personal hygiene.

Smoked fish products from current Barrel and Chorkor ovens exceeded the European Commission limit of PAHs concentration (i.e. 5 ug/g WW).

Benzo (a) pyrene values recorded from smoked fish samples from Ahotor oven (using wood ash) were lower than the EC threshold of 5 ug/g WW.

Smoked fish samples from Ahotor oven using wood ash possess no cancerous and mutagenic risk to consumers.

Consumption of fish samples from both Barrel and Chorkor ovens poses significant risk to the health of consumers.

### **Recommendations**

Fish processors who use the Ahotor oven should be educated on the need to apply wood ash on the fat collector in smoking fish for consumers.

Fish traders at the various fish landing sites should be schooled on personal hygiene regarding fish and the need to ensure clean working environment.

To further reduce the level of PAHs, studies on varying amount of wood ash used in smoking should be undertaken.

## **7. AREAS FOR FURTHER RESEARCH**

Areas for further research include:

- Assessing the level of PAHs on fatty pelagic fishes including tunas and chub mackerels. Most of these fatty pelagic fishes are consumed with the skin, making consumers prone to cancer related diseases.
- Investigating the contribution of commonly used fuelwood ash on PAHs level in smoked fishes.
- Investigating the toxicity of various packaging materials on the quality of smoked fishes in Ghana, as most traders were observed packaging smoked fishes with cement papers.



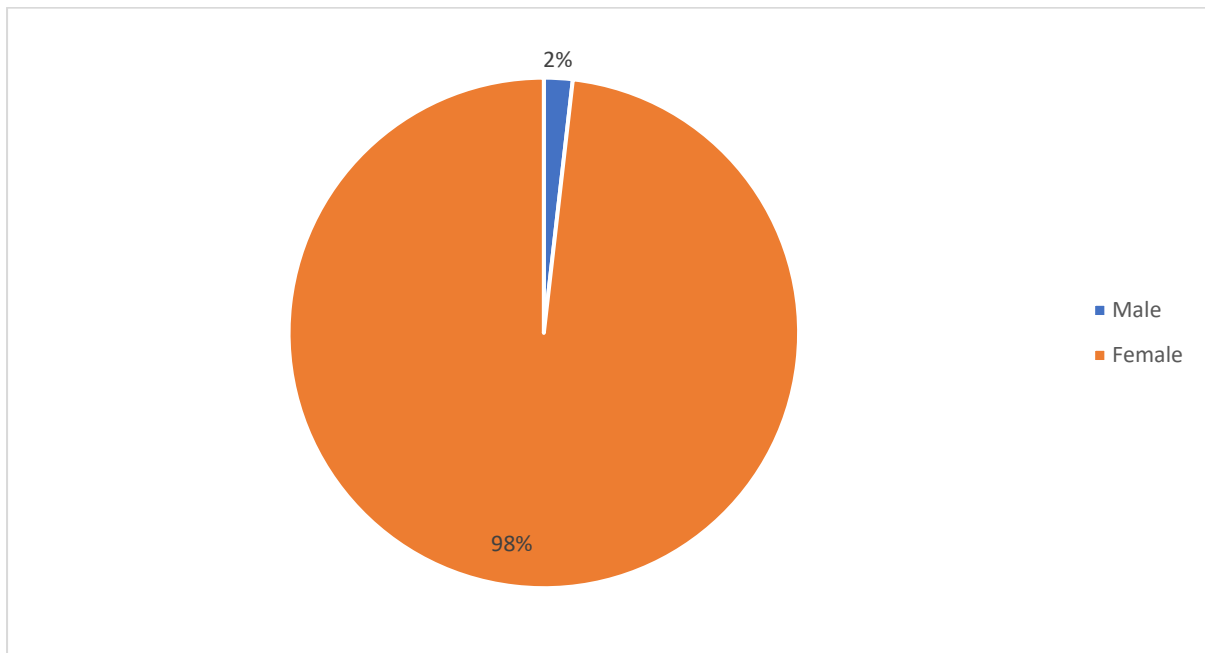
## 8. REFERENCES

- Adams, M. R. and Moss, M. O. (2003). *Food Microbiology*. Third Edition. The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF, UK.
- Adu-Gyamfi, A. (2006). Studies on microbiological quality of smoked fish in some markets in Accra. *Ghana J. Sci.* 46:675.
- Akpan V, Lodovici M, Dolara P. (1994). Polycyclic aromatic hydrocarbons in fresh and smoked fish samples from three Nigerian cities. *Bull. Environ. Contam. Toxicol.* 53:246-253.
- Akrofi, J. D. (2002). Fish utilization and marketing in Ghana: State of the art and future perspective. *Large Marine Ecosystems* 11:345-354.
- Al-Jedah JH, Ali MZ, Robinson RK (1999). The nutritional importance to local communities of fish caught off the coast of Qatar. *Nutr. Food Sci.*, 6: 288-294.
- Anyakora C, Arbabi M, Coker H. (2008). A screen for benzo (a) pyrene in fish samples from crude oil polluted environments. *Am. J. Environ. Sci.* 4:145-150.
- Baffone, W., Pianetti, A., Bruscolini, F., Barbieri, D. and Citterio, B. (2000). Occurrence and expression of virulence related properties of *Vibrio* species isolated from widely consumed seafood products. *International Journal of Food Microbiology* 54:9–18.
- Bank of Ghana. (2008). *The Fishing Sub-sector and Ghana's Economy*. Research Department, Bank of Ghana.
- Cofie, A. (2003). *Processing Environment and the microbial quality of smoked fish in the Accra and Tema Municipalities*. MPhil Thesis Department of Environmental Science, University of Ghana.
- Colakoglu, F. A., Ozen, O and Cakir, F. (2006). Microbiological quality of seafood in the Dardanelles, Turkey. *Pak. J. Biol. Sci.* 9:425-427.
- Debrah, J. S, Obodai E. A. Aheto D. W, Ameworwor D. H. A. K, and Sarpong-Baidoo, M. (2011). Analysis of the genetic diversity in coriander *Journal of Microbiology and Biotechnology Research*, 1(4), 98-205.
- EFSA. (2008). *Polycyclic Aromatic Hydrocarbons in Food*. Scientific Opinion of the Panel on Contaminants in the Food Chain (Adopted on 9 June 2008) [Internet]. *The EFSA Journal*. 724:1-114; Available from:
- Essuman, K.M. (1982). The effects of hot smoke-curing on the protein and organoleptic quality of West African herrings (*Sardinella eba*) processed in improved traditional ovens. MSc. Thesis, Department of Nutrition and Food Science, University of Ghana.
- Essumang, D. K.; Dodoo, D. K. and Adjei, J. K. (2012). Polycyclic aromatic hydrocarbon (PAH) contamination in smoked-cured fish products. *Journal of Food Composition and Analysis*, 27: 128 – 138.
- FAO. (2005). *The economic and social contribution of fisheries to gross domestic product and rural development in Ghana*. The final report of sustainable fisheries livelihoods programme submitted to Food and Agriculture Organization (FAO), Rome, Italy.
- Farhadian A, Jinap S, Hanifah HN, Zaidul IS. (2011). Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. *Food Chem.*, pp. 141-146.

- Fishery and Aquaculture Country Profiles. Ghana (FACP) (2016). Country profile Fact Sheets. In: FAO Fisheries and Aquaculture Department [Online]. Rome. Updated 2016.
- Garcia-Falcon MS, Simal-Gandara J. (2005). Polycyclic aromatic hydrocarbons in smoke from different woods and their transfer during traditional smoking into chorizo sausages with collagen and tripe casings. *Food Addit. Contam.* 22: 1-8.
- Garcia-Falcon MSG, Amigo GS, LMA Y, Villaizan MJ, Lozano SL. (1996). Enrichment of benzo (a) pyrene in smoked food products and determination by high-performance liquid chromatography- fluorescence. *J. Chromatogra.*, 753: 207-215.
- Gordon, A., Pulis, A., Owusu-Adjei, E. (2011) “Smoked marine fish from Western Region, Ghana: a value chain assessment”, WorldFish Center. USAID Integrated Coastal and Fisheries Governance Initiative for the Western Region, Ghana. 46pp.
- Kawamoto, H.; Horigoshi, S. and Saka, S. (2007). Pyrolysis reactions of various lignin model dimmers. *Journal of Wood Science*, 53: 168 – 174.
- Ministry of Food and Agriculture (MOFA) (2017). Fisheries. Accessed from [www.mofa.gov.gh/site](http://www.mofa.gov.gh/site)
- Nyarko, E.; Botwe, B. O. and Klubi, E. (2011). Polycyclic Aromatic Hydrocarbons (PAHs) levels in two commercially important fish species from the coastal waters of Ghana and their carcinogenic health risks. *West African Journal of Applied Ecology*, 19: 53 – 66.
- Plahar, W. A., Nerquaye-Tetteh, G. A. and Annan, N. T. (1999). Development of an integrated quality assurance system for the traditional *Sardinella* sp. and anchovy fish smoking industry in Ghana. *Food Control*, 10 (1): 15 – 25.
- Rengarajan, T., Rajendran, P.; Nandakumar, N.; Lokeshkumar, B.; Rajendran, P. and Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine*. 5(3): 182–189.
- Simko P (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *B: Analytical Technologies in the Biomedical and Life Sciences, J. Chromatogra.*, 770: 3-18.
- Stolyhwo, A. and Sikorski, Z. E. (2005). Polycyclic aromatic carbons in smoked fish – a critical review. *Food Chemistry*, 91: 303 – 311.
- Sustainable Fisheries Management Project (SFMP). (2015). Post-harvest Process Characterization Studies and Needs, Assessment Report on Fish Smoking and Salting of Fish. June 2015, Accra – Ghana.
- Tongo, I.; Ogbeide, O. and Ezemonye, L. (2017). Human health risk assessment of polycyclic aromatic hydrocarbons (PAHs) in smoked fish species from markets in Southern Nigeria. *Toxicology reports*, 4: 55 – 61.
- Visciano P, Perugini M, Amorena M, Ianieri A. (2006). Polycyclic aromatic hydrocarbons in fresh and cold-smoked Atlantic salmon fillets. *J. Food Prot.* 69:1134-1138.

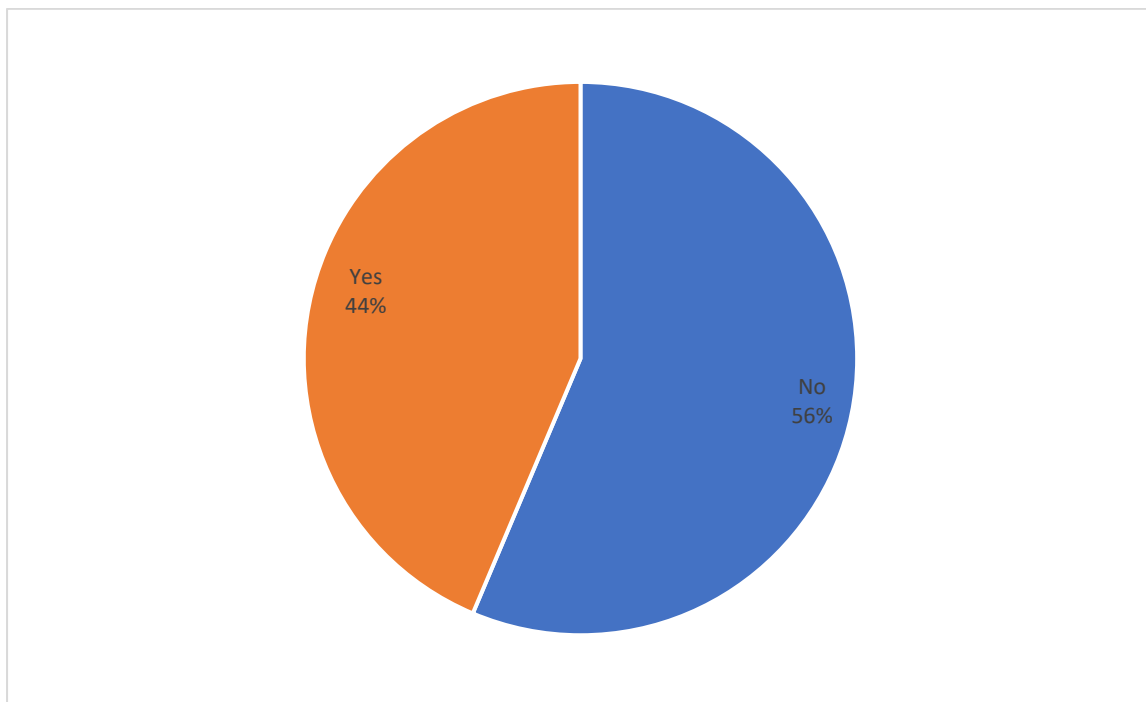
## APPENDIX. RESPONSES FROM QUESTIONNAIRE

More than half of the respondents were females (98 %) with 2 % of respondents being males, which suggest that fish trade or processing is mostly undertaken by women (Figure 9).



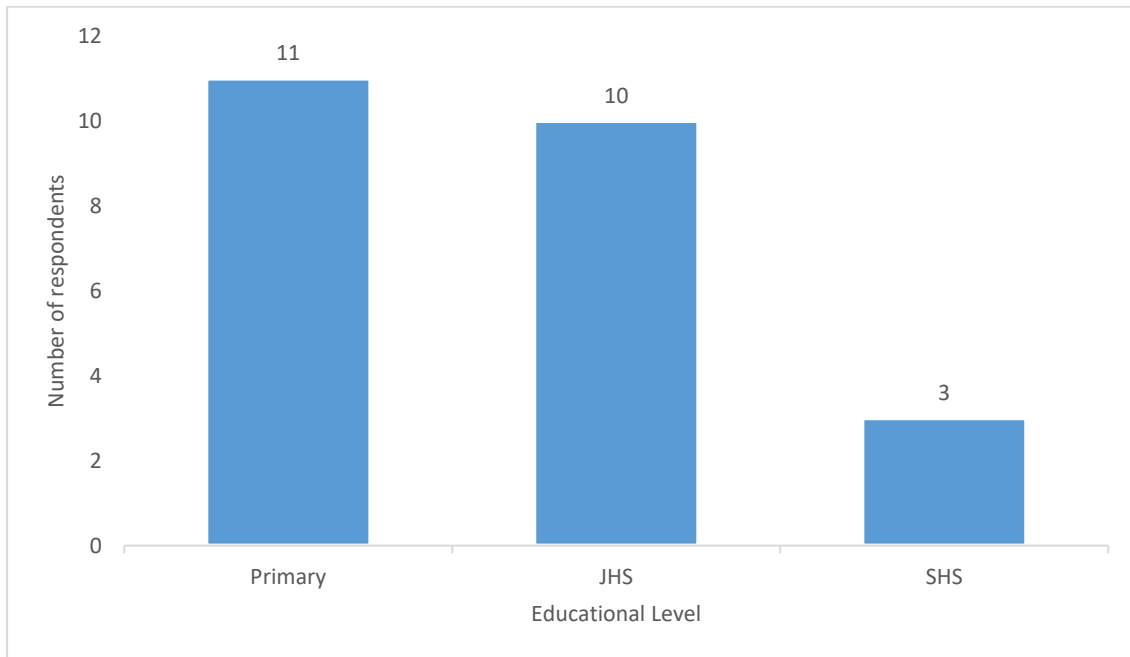
**Figure 9. Gender of respondents**

A majority of the respondents (56%) were found to be without formal education with relatively lower percentage (44%) having received formal education (Figure 10). This observation supports earlier claims that education is unpopular among fish processors or traders.



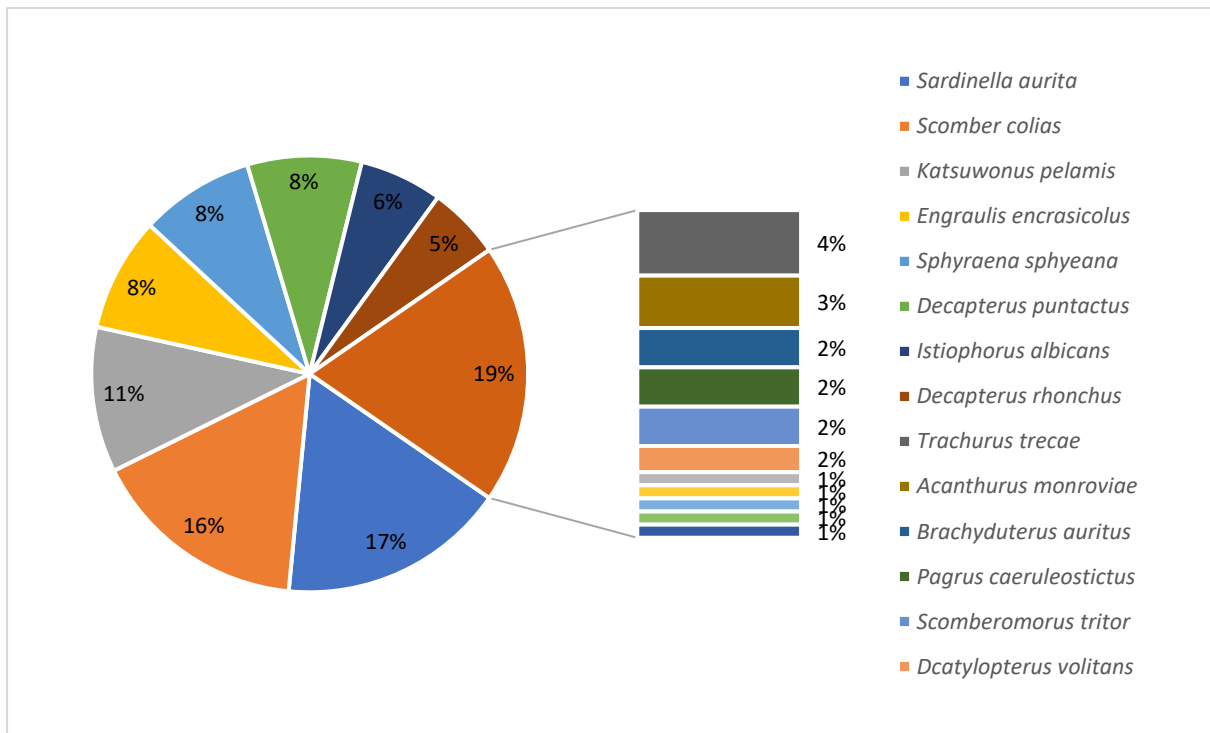
**Figure 10. Formal education status among respondents**

However, in relation to respondents with formal education, majority (11 out of 21) are with primary while a few (3 out of 24) are with second cycle education.



**Figure 11. Educational level among educated respondents**

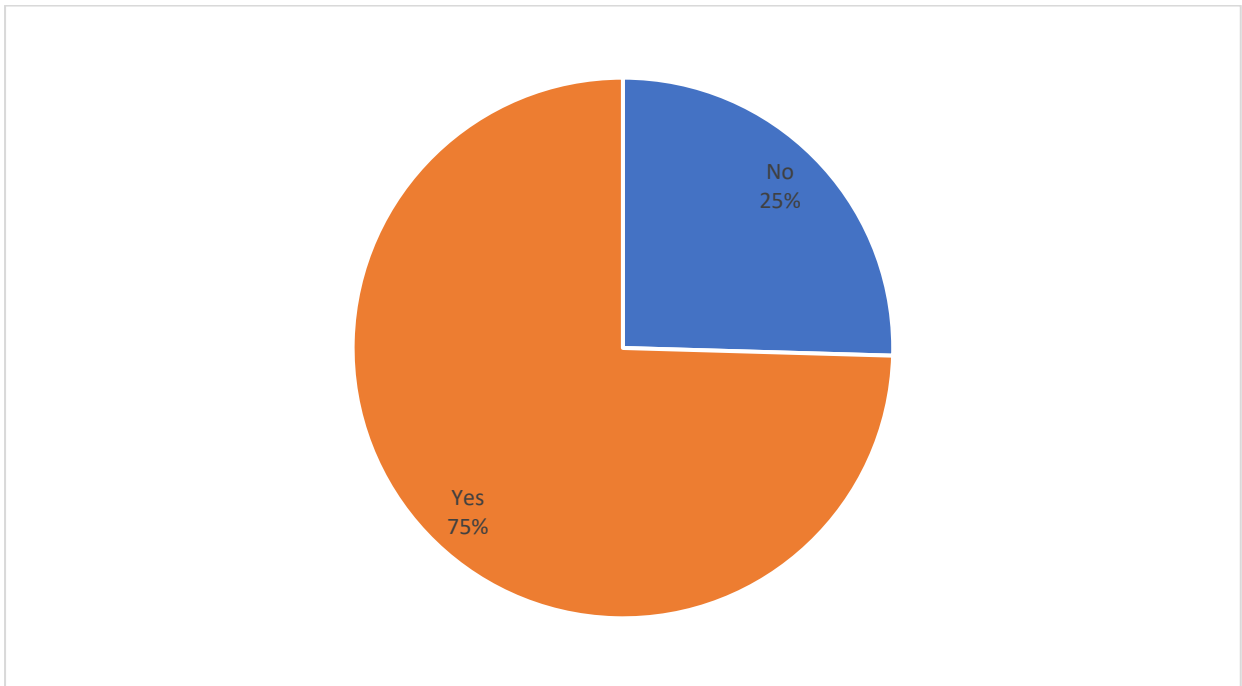
Out of nineteen (19) fish species identified to be traded at the various markets visited, *Sardinella aurita* (17%) was the dominant fish species, followed by *Scomber colias* (16%), *Katsuwonus pelamis* (11%), *Engraulis encrasicolus* (8%), *Sphyraena sphyraena* (8%) and *Decapterus puntactus* (8%) as shown in Figure 8.



**Figure 12. Fish species recorded from fish markets along the coast of Ghana**

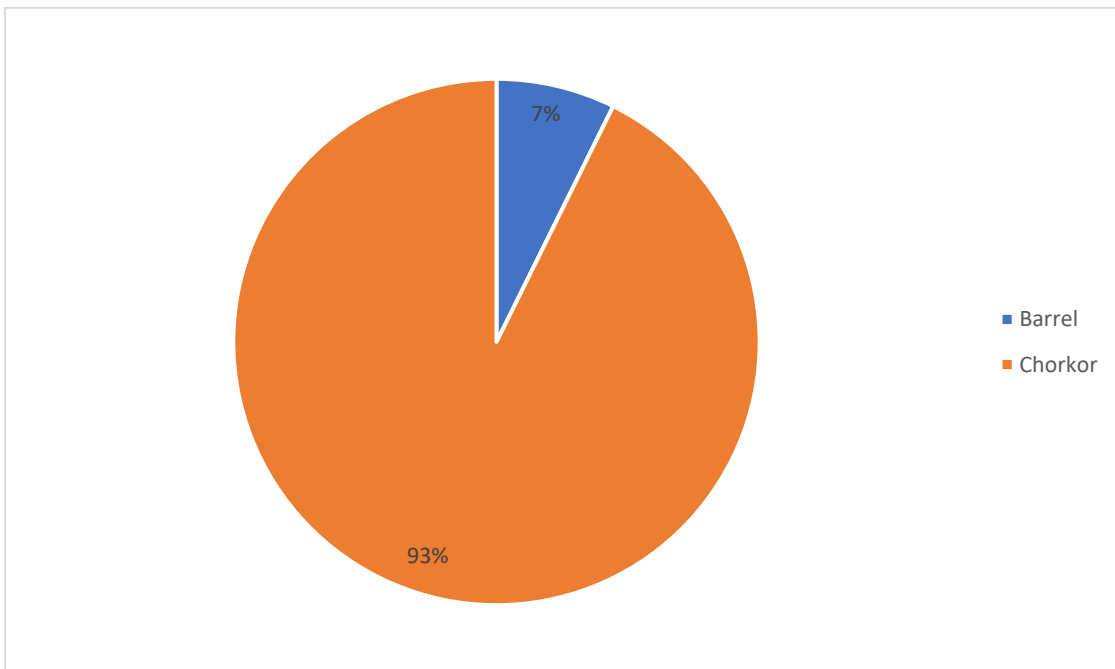
A majority of the traders (75 %) were found to be fish processors with minority (25 %) categorized as fish traders only (Figure 13). This observation was due to the fact that most of

the fish traders were introduced to the fish processing through different channels (e.g. relatives, friends and others).



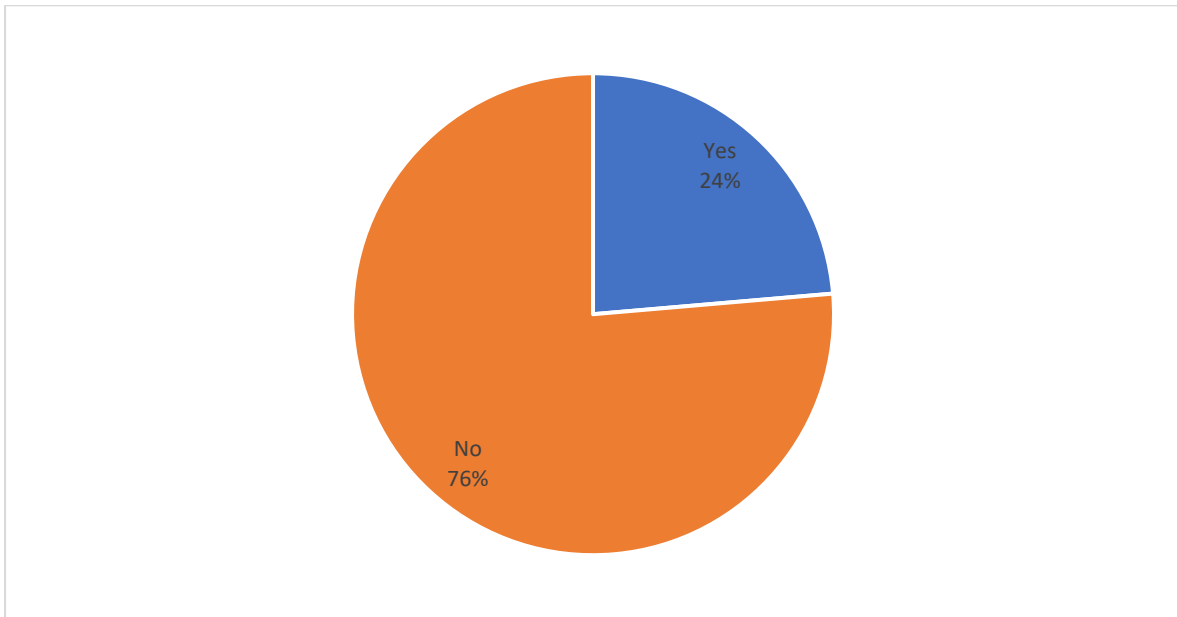
**Figure 13. Percentage of fish trader respondents also identifying as fish processors**

Most of the fish traders (93 %) who are also fish processors admitted smoking fish with the Chorkor smokers while a few (7 %) still use the Barrel oven (Figure 14). The use of Barrel oven is largely restricted to big sized fish species, hence its unpopularity among fish processors as majority of fish species are mostly small sizes.



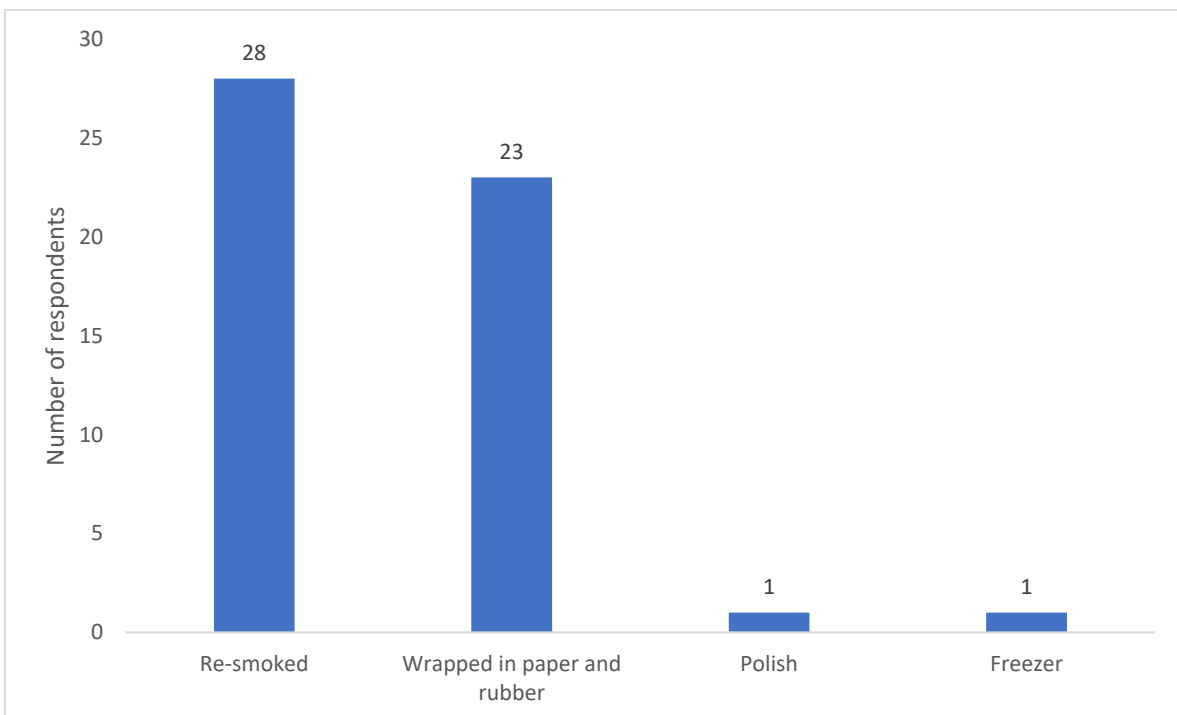
**Figure 14. Smoking ovens used by fish trader respondents who are also fish processors**

Regardless of the fishing season (i.e. whether lean or peak), more than half of the respondents (76%) admitted not being able to sell all the smoked fish products in a day with minority of respondents (24 %) responding in the affirmative (Figure 15).



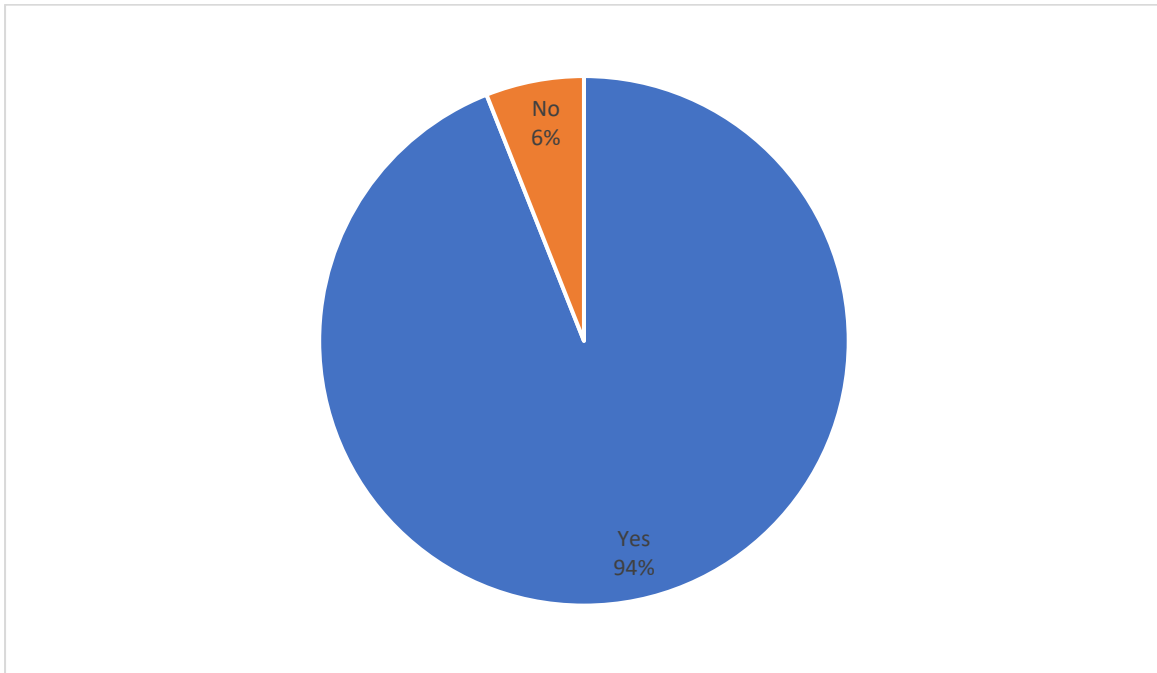
**Figure 15. Percentage of respondents able to sell all their smoked fish products in a day**

Unable to sell all the smoked fish products, most of the respondents indicated that they re-smoke the smoked fish samples for the next day (Figure 16).



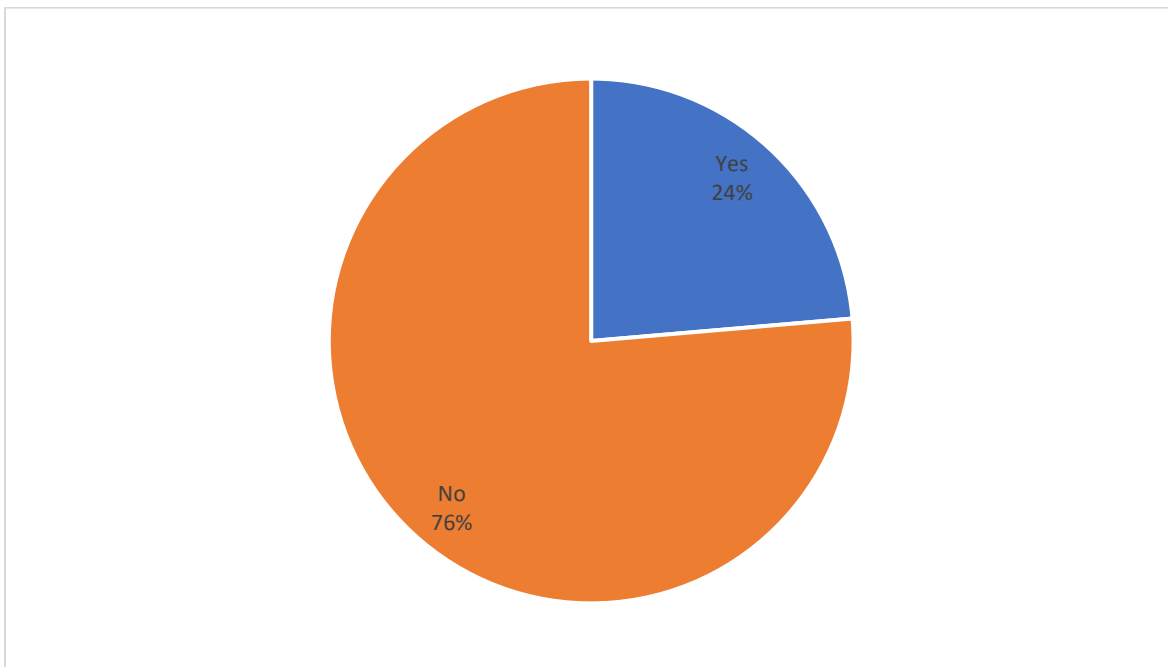
**Figure 16. Preservation techniques for unsold smoked fish products by respondents**

More than half of the respondents (94 %) reported that consumers prefer smoked fish product to frozen fish products, largely due to its high storability characteristics (Figure 17).



**Figure 17. Percent of respondents reporting that consumers prefer smoked fish products to frozen fish**

Most of the respondents (76 %) reported not having heard of PAH in smoked fish products and its related carcinogenic risk (Figure 18). Minority of respondents (24 %) responded in the affirmative, mostly due to the community awareness programs by some NGOs, particularly SNV, Ghana.



**Figure 18. Percent of respondents aware of carcinogenic risk of smoked fish products.**