UNIVERSITY OF CAPE COAST

GOOD MANUFACTURING PRACTICES (GMP) TO IMPROVE THE SAFETY AND QUALITY OF SMOKED FISH

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BY

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A Thesis submitted to the Department of Molecular Biology and Biotechnology of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Molecular Biology and Biotechnology

SEPTEMBER, 2023

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature: …… Date: ……

Name: ……

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: …… Date: …….

Name: Dr. Cynthia A, Adinortey

Co-Supervisor's Signature: …… Date: ……

Name: Dr. Nazir Kizzie-Hayford

ABSTRACT

Current methods employed in traditional fish smoking as well as the implementation of good manufacturing practices (GMP) and Hazard Analysis Critical Control Points (HACCP) were investigated to identify microbial contaminants along fish processing value chain and to improve the safety and quality of traditional fish processing in Cape Coast. The total viable bacterial, coliforms, *E. coli*, and fungal counts were determined using the pour plate method. Physicochemical and nutritional qualities were also assessed by measuring pH, texture, brix, weight, browning index, chemical quality was analysed for peroxide value, histamine and polyaromatic hydrocarbons (PAH) and nutritional qualities were also assessed by conducting proximate analysis. The total viable bacterial counts (TVBC) of the fresh fish were in the range of 4.18±0.02 – 4.20±0.13 (log CFU/g), coliforms, *E.coli* and fungal counts for the fresh fish were also 2.84±0.09 –2.72±0.09, 1.59±0.07 –1.11±0.10 and 3.86±0.08 - 3.65±0.07 (log CFU/g) respectively. After washing the fish with tap water, microbial count reduced to 3.60±0.03 – 398±0.03 (log CFU/g) for TVBC, 2.24±0.09 – 2.39±0.12 (log CFU/g) for coliforms, 1.09±0.00 – 0.85±0.21(log CFU/g) for *E.coli*, and 2.84±0.09 – 2.90±0.08 (log CFU/g) for the fungal count. Smoking led to a reduction in < 1 log CFU/g counts for coliforms, *E. coli*, and 1.09 ± 0.12 – 1.39 ± 0.12 log CFU/ g fungal count. Implementation of GMP/HACCP led to lower counts of TVBC, coliforms, *E. coli*, and fungal counts below detection limits (<1.0 log cfu/g) of smoked fish. Histamine levels were in the range of 14.67 ± 1.92 mg/kg to 17.55 ± 5.9 mg/kg, were within the acceptable limits while peroxide values in the range of 48.26–56.85 meq/kg to 22.63–27.65 meq/kg remained higher than recommended levels with no detection of PAH4 levels in the smoked fish. This study has revealed that applying HACCP through the observation of good manufacturing practices, minimum handling, as well as segregation of raw materials from finished products, can improve the microbial quality of smoked fish.

KEYWORDS

Food safety

Good Manufacturing Practices (GMP)

Hazard Analysis and Critical Control Points (HACCP)

Frozen mackerel

Traditional fish smoking

Microbial contamination

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DEDICATION

To my husband Mr. Henry Kofi Foli and my children Sesinam Abla Foli and Aseye Yawa Foli.

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CHAPTER ONE

INTRODUCTION

Background Study

Seafood are considered to be one of the healthiest foods on earth because it is a good source of protein, and healthy fats, and also play a vital function in fighting hunger and malnutrition (Food and Agriculture Organizations for the United Nations, 2017). Globally, fish represent 17 percent of over-all animal protein, and demand keeps increasing (Nations, 2020). In the coastal regions of West Africa, traditional fishing is practiced in rivers, lakes, ponds, and seas (Adeyeye *et al*., 2015). The consumption of fish per capita in Ghana is estimated at 25kg, which is higher than the average annual capita of 18.9kg and 10.5kg for the world and Africa respectively (Onumah *et al*., 2020). To sustain the present per capita levels of consumption, Ghana imports nearly 191.000 metric tons of fish yearly from other countries, which merely satisfies approximately 50 percent of the total national fish requirement of about 420,000 metric tons (Quaye, 2018). The main species of fish imported into Ghana are mackerel and sardines/sardinella (Taylor, 2022). Preservation methods like freezing, drying, salting, and smoking helps to reduce high post-harvest losses during the major fishing season which is from July to September each year. Additionally, these preservation methods make fish available during the lean season.

Fish is vital component of a Ghanaian diet, and the cost is affordable; almost 75% of the total catch in the country is locally consumed. In addition, the consumption of whole small fish (sardines, anchovies) is beneficial as it represents a high nutritional density of proteins, vitamin A, D, and B_{12} (Food

and Agriculture Organizations for the United Nations, 2017; Kawarazuka & Béné, 2011).

Statement of the Problem

Preservation methods like freezing, chilling, smoking, drying, frying, and fermentation are procedures employed to reduce post-harvest loss of seafood quality caused by microbes and enzymes, physical injury, and chemical reactions (Sigurj & Emil, 2010). In Ghana, marine and fresh water fish are preserved by smoking, sun drying, salting, and fermentation (Asiedu *et al*., 2018; Plahar *et al*., 1991). Smoking is the oldest food preservation procedure and is still commonly employed in fish processing in Ghana. Food smoking is still significant in developing countries since modern alternatives require electricity which is expensive for the local folks (Ogbadu, 2014).

According to Hasselberg *et a*l., (2020), 80% of fish landing in Ghana are processed traditionally and fish is either wet hot smoked or dry hot smoked. Hot smoking is the most preferred procedure employed in fish processing in Ghana with the smoking intensity being modulated by adjusting the quantity of firewood to achieve optimal fish cooking weight, flavor, colour, and aroma as desired (Asiedu *et al*., 2018; Sakyi *et al*., 2019). Additionally, marine fish like mackerel is mostly hot-smoked to yield a moist (40%-50%) product and most importantly, to attract a high market premium. Smoke from wood reduces moisture, coagulates proteins, and inhibits bacterial growth, acting as a physical barrier against surface contaminants (Puke & Galoburda, 2020).

Despite the antimicrobial effects of smoke on fish, safety concerns have been raised about contaminated fish in the Ghanaian local market

(Aboagye *et al.,* 2020; Ndaa Norbert, 2014; Obodai, *et al.,* 2011). The hygiene and safety issues are mainly from poor handling and unclean processing environment. For most localities, traditional fish processors in Ghana operate in the open or under shelter, which are dusty and without a proper refuse disposal system. These conditions contribute to contaminating fish through the processing stages, including post-processing. The unsanitary conditions of the processing facility, and environment can contribute to the growth of bacteria, yeast, and moulds in smoked fish. Then also, high initial number of microbial contaminants in smoked fish shortens storage time.

Potential hazard associated with smoked fish include physical, biological and chemical contaminants. While physical contaminants are foreign materials (plastics, pieces, hair, shell fragments) from the harvesting environment, packaging, transporting or storage (Laly et al., 2022), biological contaminants include microbes such as *Clostridium botulism*, *Listeria monocytogenes*, *E. coli, Staphylococcus aureus, Klebsiella* spp., according to Lin *et al*., (2008). Chemical contaminants are toxins (histamine) produced by microbes and polycyclic aromatic hydrocarbon compounds as a result of smoke generated during fish smoking.

Hygienic conditions for fish smoking in Ghana are still sub-optimal whereas the requirements for the smoking process can sometimes become compromised to exploit economic gains. Although the USAID Sustainable Fisheries Management Project (SFMP) through Ghana Fisheries Commission's (GFC) has developed hygiene practice programs by training artisanal fish processors (Addo Opare, 2019), there are still a number of fish processors who still process fish under unsanitary conditions and without

adhering to hygienic practices. This could probably be because of limited start-up money, low level of education and lack of institutional support contribute to food hygiene practices among local fish processors (Hasselberg, Aakre, *et al*., 2020; Sakyi *et al*., 2019). To be exact, the few fish processors who have received training on food safety complain of lack of financial support in implementing proposals from quality experts. It has become necessary to develop and implement food hygiene and safety protocol that is simplified to a level that can easily be integrated into traditional fish processing to improve smoked fish safety and quality.

Research Questions

1. What are the stages and gaps in the most frequently used method for fish smoking in Ghana?
2. What is the effect of the traditional fish smoking method on the safety and quality of smoked fish?
3. What is the effect of applying GMP and HACCP on the safety and quality of smoked fish?

Main Objective

The primary aim of this study is to assess the impact of implementing a basic Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) to improve the safety and quality of smoked fish.

Specific Objectives

The objectives of this study was to

1. identify gaps in fish smoking process that must be addressed to improve the safety and quality of smoked fish.

2. determine the impact of fish smoking procedure on the safety and quality of smoked fish.
3. determine the impact of applying GMP/HACCP procedure on smoked fish quality.

Hypothesis

The main hypothesis of the research is that, the implementation of a basic GMP and HACCP will improve the quality and safety of smoked fish.

Significance of Study

Even though fish and fishery products are part of a typical Ghanaian dish and contribute additional nutritional requirements for children, post-harvest losses of fish affect food security. The hygienic conditions for fish smoking in Ghana are still sub-optimal whereas the requirements for the smoking process can sometimes become compromised to exploit economic gains. Moreover, fish consumption benefits outweigh the potential hazard associated with fish consumption. Report by Aboagye *et al*., (2020) revealed that awareness of the unsatisfactory microbial safety of artisanal fish in the Ghanaian market is needed. Statistical reports (Kleter, 2004) showed that the number of countries including Ghana exporting fish to the EU has declined substantially. There is a need for constant checks on the safety and quality of smoked fish to ensure its safety for human consumption.

Good Manufacturing Practices and HACCP approach to fish smoking and handling will not only improve fish safety and quality but also provide traditional fish processors with the needed knowledge and skills in food safety. Also, a GMP and HACCP document will be developed for fish handling from the fresh state through the processing and post smoking steps in Ghana. It is

also expected that the quality and safety of smoked fish will improve upon the implementation of a basic GMP and HACCP procedure.

Organization of Study

This thesis is divided into five chapters. The first chapter serves as an introduction and provides the research background. In the second chapter, the literature review focuses on Ghana's fish production capacity, current fish smoking practices, the hazards associated with smoked fish, and nutritional benefits obtained from fish consumption. The third chapter describes the study area, the microbiological, physicochemical analysis performed on the fish samples. The fourth chapter describes the results obtained in identifying gaps in traditional fish smoking, the effects smoke on the microbiological and physicochemical quality of smoked fish and the impact of applying a basic GMP/HACCP on the microbiological and physicochemical quality of smoked fish. Finally, in the fifth chapter, the research findings are discussed, including the impact of implementing good manufacturing practices on the fish smoking process. Recommendations and conclusions are also provided.

CHAPTER TWO

LITERATURE REVIEW

Ghana's Fishery Sector

The fishery sector is crucial to Ghana's economic development, providing employment, foreign exchange revenues, safeguarding food security, and poverty alleviation. According to Sarpong *et al*. (2005), the significance of the fishery sector cannot be underestimated. Around 20 percent of the active labor force (2.7 million people) is employed in the fishery sector, including women who are involved in processing and distribution (Akpalu *et al*., 2018). Tall and Failler (2012) also emphasize that the fishery sector is essential for supporting the livelihoods of many rural folks in Ghana. Fish are largely obtained from the ocean and inland (aquaculture) (Tall & Failler, 2012). While freshwater fish are mainly from the Volta Lake, fishponds, and lagoons, aquaculture holding systems are mainly floating cages, earthen ponds, and concrete tanks (Amenyogbe *et al*., 2018).

Marine fisheries

Ghana has a maritime shoreline of approximately 343.8 miles and an over-all inland area of almost 24,000 km^2 which sustains the marine fishing industry (Tall & Failler, 2012). About 304 coastal landing sites are located in 189 fishing communities, employing nearly 1.5 million people (Sarpong *et al*., 2005). The marine sub-sector played a crucial role in the annual domestic production between 2010 and 2017, amounting to approximately 314,000 tons, which constituted about 70% of the overall fish supply (Alabi-Doku *et al*., 2017).

Fish resources from the marine coastline are grouped into four; namely small pelagic, large pelagic, demersal, and mollusc and crustaceans (Sarpong *et al*., 2005). Although the small pelagic groups are mostly abundant and cover a wide range, round and flat sardinella. anchovy and chub mackerel are of great economic value (Akpalu *et al*., 2018). The demersal fish species are mostly Seabeams, cassava fish, Snappers, Groupers, Cuttlefish, Lobster, and shrimp. The large commercial pelagic are the yellowfin, skipjack and bigeye (Alabi-Doku *et al*., 2017).

Inland sector

Inland fishery are classified to be fresh water inland catch and culture based fisheries and aquaculture (Cobbina, 2010). The Lake Volta is a habitat for about 140 fish species and its landing is mostly tilapia (38.1%), *Chrysichtys* spp. (34.4%), *Synodrantis* spp. (11.4%), *Labes* sp. (3.4%), *Mormyrids* sp. (2.0%) *Heterotis* spp. (1.5%), *Clarias* spp. (1.5%), Schilbeide spp. (1.4%), odaxothrissa *mento* (1.4%), *Bagrus* spp. (1.35) and Citharinus spp. (1.2%) and the rest which are less than 1% include *Alestes* spp., *Brycinas* spp., *Distichodus* spp., and *Gymnarchus* spp., *Hydrocynus* spp., and *Lates niloticus*, (Tall & Failler, 2012). However, aquaculture production accounts for 80 percent of tilapia species and 20 percent for catfish species (Food and Agriculture Organisation of the Untied Nations (FAO), 2013).

Importance of Fish

Fish plays an important role in ensuring food security and preventing malnutrition worldwide, according to the Food and Agriculture Organisation of the United Nations in 2017. They are not only responsible for recycling essential nutrients required by other aquatic species, such as algae, to survive

in the ecosystem, but they also hold significant economic and industrial value. Millions of people involved in culturing, harvesting, processing, and trading fish locally and globally depend on the industry for employment including 820 million families and their dependents according to High Level Panel of Experts (HLPE) in 2014. Moreover, fish, whether farmed or captured from marine, coastal, or freshwater sources, are the basic source of protein for many developing countries. As a result, their consumption and utilization have been increasing globally, as stated by Asiedu in (2018) and Tall & Failler in (2012).

Fish nutritional value

Fishes are known to be cold-blooded water vertebrates belonging to upper-class Pisces naturally having gills, fins, and a streamlined body (Pal *et al.,* 2016). Fish and fishery commodities provide the best protein, fat, and vitamin and omega-3 fatty acids in fatty fish promote optimal brain activity while reducing the occurrence of heart attacks, strokes, and osteoporosis. (Alhassan, *et al*., 2013). For this reason, fish like all other seafood are essential commodity in international trade due to their increasing consumption demand (Lamy & Szejda, 2020; Puke & Galoburda, 2020).

Fish is deemed as a balanced diet because it contains the required nutrients to prevent malnutrition, especially in Africa, and also fish inclusion in the meals of infants, children, and pregnant, and lactating mothers helps in brain development and the central nervous system (Kawarazuka & Béné, 2011; Yazew *et al*., 2020). Above all, fish muscle contains about 60-80% water, 18-20% protein, 1-2% carbohydrates, and 8-20% lipids that must be preserved with little or no change (Kotsanopoulos & Arvanitoyaannis, 2012; Puke & Galoburda, 2020). For these benefits, demand for fish and its products

has increased because of its fast cooking and easy digestibility (Alhassan *et al*., 2013).

Notably, worldwide utilisation of seafood per capita has increased by two folds from 10 kg in 1960 to above 20 kg in 2014 (Guillen *et al*., 2019) and is projected to go as much as 30% high from 2010 to 2030 due to its high protein (Kogbe, 2015). The high nutritional composition of seafood is vital in many countries, especially where overall protein intake is low (Guillen *et al*., 2019). In developing countries, fish is an important food commodity (Steiner-Asiedu *et al*., 1991), because it is direct source of micronutrients such as vitamin A, B, and D, and long-chain omega-3 fatty acids that contribute to food security (Elbashir et al., 2018; Pop & Frunză, 2015).

Presently, the Agriculture sector dominates the Ghanaian economy employing about 60 per cent of the labour force (FAO, 2021; Sakyi *et al*., 2015). Agriculture, predominately smallholder, traditional, and rain-fed, contributes 45-50 per cent of the GDP and about 75 per cent of the exported earnings of Ghana. The fisheries subsector represents 5 per cent of the country's agricultural GDP (FAO, 2007), where the fish reserves are from sea and inland (freshwater), coastal lagoons and aquaculture (Ndaa Norbert, 2014). And also employing the people along the fish value chain (FAO, 2007; Kawarazuka & Béné, 2011). Fish is the leading provider of animal protein in Ghana, and approximately 75 per cent of the over-all fish catch is locally utilised. Fish spices like casava fish, red snapper, sea bean, barracuda, and croaker are considered to be high premium and so attract high monetary value and are enjoyed by most households. (Taylor, 2022).

Fish Production in Ghana

Regardless of Ghana's large coastline and fresh water, fish stocks are being excessively exploited, leading to a decrease in marine fisheries (Alabi-Doku *et al*., 2017). This is mainly due to intense pressure from offshore trawlers, uncontrolled artisanal beach seining, and harvesting of juveniles from lagoons. Even the Volta Lake, which is the main source of inland fisheries, is showing signs of overfishing (Tall & Failler, 2012). Experts in the industry are concerned that the current rate of decline in fish stocks due to overfishing and illegal, unregulated, and unreported (IUU) fishing activities will make it challenging to increase domestic production in the future (Taylor, 2022). They forecast that any future increase in domestic production will come from aquaculture, but the productivity of aquaculture is yet to achieve its full potential due to several challenges (Taylor, 2022). These challenges include limited availability of fingerlings, expensive feed, lack of financial resources, and limited availability of extension services (Cobbina, 2010).

Fish Spoilage

Spoilage of food is the degradation of the food to a point that it becomes unsafe for human consumption (Getu & Misganaw, 2015). Seafood is considered perishable food products that deteriorate rapidly after catch, during handling and storage (Pal *et al*., 2016). In addition, seafood spoil at hot climates and tropical areas where cold preservation resources are expensive (UNIDO, 2015). Coupled with post-harvest losses occurring along the value chain in various degree and extent. According to Likongwe *et al*., (2019) post-harvest loss is explained as decreased in quantity, and quality that affects monetary worth of fish in the value chain. This is considered nutritional loss

since a decomposed fish is deemed unsafe for human ingestion and the revenue of persons involved in the value chain and availability of fish as food is negatively affected (Getu & Misganaw, 2015). This, therefore, represents a significant food insecurity.

Notably, fish spoilage occurs soon after their death due to their chemical composition (water, protein, lipid, and carbohydrate) and the nature of their habitat (Alam, 2020). The water in which fish live contains millions of bacteria that come into contact with fish through their mouth, gills, and stomach. Although most of these bacteria are harmless to fish, some can cause foodborne diseases in humans (Alam, 2020). When a fish dies, the fish muscles gradually harden, and the entire body becomes stiff (rigor mortis) within a few hours of death. The hardening process can last for a couple of hours, depending on the species, temperature, and other conditions at the time of death (Gram & Dalgaard, 2002). After rigor mortis, the muscles become soft again, which is when the process of spoilage begins. At this point, autolysis sets in where enzymes responsible for biochemical changes digest fish tissue composition including lipid, proteins, and carbohydrates (Henrik & Gram, 1996).

The enzymes present in the muscle, viscera, and digestive tract, as well as those produced by bacteria, break down the muscles into smaller components. Then also spoilage bacteria (eg. *clostridium* spp) enter the muscles through the skins and gills, where these bacteria breakdown the fish cells and use the necessary energy to grow. This breakdown results in the production of acids in the muscle, which attracts spoilage bacteria from the surface, gill, and gut resulting in increased bacterial activity (Erkmen &

Bozoglu, 2016). This bacterial activity causes a heavy slime to form on the skin and gills, along with unpleasant odor, and softened muscle tissue, changes in color, texture, and off-flavors, (Erkmen & Bozoglu, 2005). Autolysis sets in shortly after the death fish, then the fish skin becomes permeable to bacteria at the same time releasing free fatty acids, amino acids, and simple sugars which provide a rich medium for bacteria to grow (Aberoumand & Baesi, 2020; Sikorski & Kolodziejska, 2002).

Microbial fish spoilage

The microorganisms present on freshly caught fish are primarily influenced by the microbial variety of the water in which they reside, according to Henrik and Gram (1996). These microorganisms can be found on the fish's external surfaces, such as their skin and gills, as well as in their intestines, both while they are alive and after they have been caught. The safety of fish and fishery products can be affected by various factors, such as the water used for washing the fish, the ice used for preservation, the equipment used during operations, and the ingredients added during food processing. This is especially problematic in situations where primary food manufacturers and workers have limited access to safe or potable water.

Water also inhabits food spoilage microorganisms including *Bacillus*, *Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *Alcaligenes faecalis*, *Escherichia coli*, *Proteus*, *Pseudomonas* spp., and *Aeromonas* spp. Water from ponds lakes, rivers, dams, and rain also contain pathogenic microbes including *Aeromonas* spp., *Campylobacter* spp., pathogenic *E. coli*, *Salmonella*, *Shigella* spp., *Vibrio* spp., *Yersinia* spp., and intestinal parasites (Erkmen & Bozoglu, 2005; Ray & Bhunia, 2013). Furthermore, human, agricultural, and animal

waste contribute to water contamination (Cabral, 2010). Likewise, fish caught is also subjected to contamination from their holding containers (plastic, wood or metal).

World Health Organisation, (2019) recommends only potable water in food processing, however, water circulated for reuse must be maintained, and handled under conditions not risky to processed food. Every so often, microorganisms of the genera *Flavobacterium*, *Shewanella*, *Aeromonas*, *Moraxella*, and *Pseudomonas* are associated with seafood. Other organisms are *Acinetobacter* spp., *Bacillus* spp., *Cytophaga* spp., *Vibrio* spp, *Coryneform* spp, *Enterobacteriaceae* spp, and *micrococcus* spp. (Elbashir *et al*., 2018; Pal *et al*., 2016). Most fish related pathogens are either indigenous freshwater habitats or those associated with water pollution (Novoslavskij *et al*., 2016). The population of organisms occurring on the skin and gills vary between 102 -107 CFU/g (colony forming units) (Pal *et al*., 2016). However, fish muscle contains few bacteria before handling and filleting (Laein, 2018; Safaeian & Khanzadi, 2018), but filleting done by hand, knives and cutting board or surfaces are sources of microbial contamination to fish flesh from the surface microflora (Kolodziejska *et al*., 2002; Sudheesh *et al*., 2013).

Chemical fish spoilage

Lipid oxidation is one of the major causes of spoilage and deterioration of fish, red meat and poultry tissue structure (Wu *et al*., 2022). Lipid oxidation leads to quality loses through the production of off-flavour and odour, low nutritional value and shortened shelf life (Secci & Parisi, 2016). Lipid oxidation becomes a quality measure at <0°C where spoilage by microbial activity is not and it begins just after fish death (Ólafsdóttir *et al*., 1997). Fish

oil contains long chains of poly unsaturated fatty acid (PUFAs) that react with oxygen causing the development of off-flavours and aromas which is often referred to as rancidity. The stability of omega-3 fatty acids has been a main interest for fish lipids in food nutrition and other related research (Kong *et al*., 2011). Primary products of fatty acid oxidation such as lipid hydroperoxides do not affect the fish flavour, however, volatile secondary oxidative compounds (aldehydes, polymers, and malondialdehyde) that are formed through the breakdown of lipid hydroperoxide give rise to off aromas and rancid flavours (Ashton, 2002; Ladikos & Lougovois, 1990). Several methods have been developed to determine the presence of both primary and secondary oxidative compounds but peroxide value (PV) and thiobarbituric acid (TBARs) are commonly used. The suitability of each method depends on the type of product, processing procedure and storage conditions (Hultin, 1994; Ladikos & Lougovois, 1990).

Biogenic amines (BA), are small molecular weight compounds, including tryptamine, tyramine, histamine, 2-phenylethylamine, cadaverine, spermine and spermidine formed in foods by microbial decarboxylation of the amino acids (Santos, 1996; Zhai *et al*., 2012). Biogenic amines perform important metabolic activity in living cells by regulating nucleic acid and protein synthesis (Santos, 1996), but are also present in varied foods like fish and meat, dairy, and other fermented products (Ahmed *et al*., 2012; Santos, 1996). Scombroid fish species (tuna, mackerel), and non-scombroid species like sardines, herring, anchovies, etc) have elevated levels of histidine naturally in their fish tissue (Ruiz-Capillas & Herrero, 2019; Visciano *et al*., 2014). Conversely, microorganisms and free amino acids can decarboxylase

under favourable conditions causing biogenic amine production (Özogul & Özogul, 2020; Park *et al.*, 2017). Moreover, enzymes including histamine, putrescine, agmatine and cadaverine are produced when microorganism breakdown protein in fish (Weremfo *et al*., 2020; Zhai *et al*., 2012). Amongst other types of biogenic amines, histamine is the most frequently reported (Visciano *et al*., 2014).

Histamine is barely detected in freshly caught fish, but its elevation indicates the decomposition of raw materials, microbial contamination, and poor processing conditions and storage (Ruiz-Capillas & Herrero, 2019). Microorganisms are innately found in the gills, guts, and skin of live fish and starts to grow once fish is dead. Histamine forming bacteria (*Morganella morganii*, *Klebsiella* spp., *H, alvei*, *Serratia* spp., *Enterobacter* spp., etc) grow rapidly at moderately abused temperatures (Kim *et al.*, 2001; Visciano *et al*., 2014). Hence, elevated levels of histamine in fish compromise the quality and safety the final products (Debeer *et al*., 2021). Consequently, the determination of histamine levels in fish is regarded as a quality index and it is a vital tool for sanitary surveillance (Zhai *et al*., 2012).

International organizations and many countries have established maximum levels for biogenic amines (Bilgin & Gençcelep, 2015). Food and Agriculture Organization/World Health Organization (FAO/WHO) committee, on the public health risk of histamine noticed no adversarial effect after a sum of 50mg of histamine was consumed (Debeer., *et al*., 2021). However, the European Union (EU) satisfactory level of histamine in pelagic fish species is less than 100mg/kg and a maximum level of total biogenic amine must not excide 200mg/kg in seafood (Bilgin & Gençcelep, 2015).

Fish Preservation

Food preservation is the practise of handling and treating foods in order to control its spoilage by stopping the attack and growth of microbes in food that cause food borne diseases, to avoid lipid oxidation (rancidity) and maintaining the nutritional value, texture and flavour of the food (Kumar, 2019). Fish and other seafood are known to rapidly perish after their harvest due to the pathogenic flora of the aquatic environment, and also to improper handling, and processing (Mahmud *et al*., 2018). Several preservation methods have been employed to fish and fishery products to maintain its quality. Preserving of raw fish converts it to suitable forms that can be stored for a long term, easy transportation to long distances and market distribution, and in addition providing employment along the value chain and (Mohan *et al*., 2013).

Fish preservation methods

Following the harvest of aquatic products, low temperature treatments are applied onboard to maintain their quality. These treatments involve several chilling methods such as moist ice, cold seawater, solid and liquid carbon dioxide, liquid nitrogen (Mahmud *et al*., 2018). Chilling is an effective procedure for maintaining the freshness of fish products. This is achieved by keeping the fish in a slurry of ice or melted ice that maintains the temperature around the fish at 0 – 4 ℃ (Mohan *et al*., 2013). This low temperature can delay enzymatic actions and microbial activity, effectively extending the shelf life of the fish.

Another old procedure employed in fish preservation is freezing which maintains freshness and quality for a long time (Kumar, 2019). At freezing

temperatures between 0 ℃ to minus 80 ℃, water for enzymes and microbial activity is reduced (Gram & Dalgaard, 2002; Mohan *et al*., 2013). Freezing is a long-term method employed mostly by importers, wholesalers and retailers to maintain quality of seafood (Wang *et al*., 2017).

Canning is another popular method of fish preservation that allows fish to be stored for an extended period (Fuadi *et al*., 2014). This process involves using thermal treatment at a temperature of 121℃ for 15 minutes to inactivate any microorganisms that may be present and ensure a good palatable fish product. However, it's worth noting that temperature susceptible vitamins like riboflavin, niacin, and thiamine are destroyed during sterilization (Abraha *et al*., 2018). In addition, fish can be processed through various heating methods such as grilling, baking, frying, boiling, cold, and hot smoking. These methods enhance the taste and flavor of the fish, as well as prolong its shelf life (Mahmud *et al*., 2018).

Fish smoking can be accomplished using two methods such as hot or cold smoking. Hot smoking is carried out at temperatures ranging from 30ºC to 90ºC, while cold smoking is done at room temperature or temperatures between 20-30ºC (Andhikawati & Pratiwi, 2021). Lower temperatures are used in the cold smoking method to prevent the fish from cooking too quickly and to retain its nutritional value. This process takes a longer time, depending on the size of the fish, and the cold smoked fish can be stored for a longer period (Birkeland *et al*., 2004). The maximum moisture content of smoked fish reaches 60% due to the drying that occurs in the fish meat (Erkmen & Bozoglu, 2016). During the smoking process, raw materials such as wood and coconut shells are commonly used and can affect the quality of the smoked

fish, including its taste, color, and antimicrobial properties (Baten *et al*., 2020; Belichovska *et al*., 2019). Additionally, liquid smoke obtained from the condensation of combustion vapors containing carbon, organic acids, phenols, and other compounds can also be used to achieve fish smoking (Belichovska *et al*., 2019; Huong, 2014).

Traditionally, fish preservation by smoking goes way back in history (Huong, 2014; Sakyi *et al*., 2019; Stołyhwo & Sikorski, 2005). Food smoking is still significant to developing countries since modern alternatives require electricity which is expensive for the local folks (Ogbadu, 2014). Smoke and heat produced by incomplete combustion of wood or sawdust impart flavour and colours as well as increase the storage life of fish (Goulas & Kontominas, 2005; Hokkanen *et al*., 2018). Antimicrobial and antioxidant constituents of smoke have been studied extensively (Baten *et al*., 2020). Smoke contains vaporized chemical compounds like formaldehyde, and acids that inhibit the growth of many microorganisms, and limit oxidation reactions (Goulas & Kontominas, 2005; Huong, 2014; Kotsanopoulos & Arvanitoyaannis, 2012). Moisture level is reduced to a minimum through the first hour of fish smouldering while the dehydrating effect of heat and smoke sterilizes the fish and halt enzyme activity in the fish tissue (Ogbadu, 2014).

An additional preservation effect is attributed to salt addition at the onset of fish processing. The preservation effect of salt is purposely owed to decrease in water activity which hinders the proliferation of food spoilage microbes on the fish skin (Goulas & Kontominas, 2005). There are many advantages of fish smoking. Fish smoking enhances flavour, increases utilization of smoked fish in sauces and soups as well as prolonged shelf-life.

It reduces post-capture loss in bumper harvest and permit storage in the lean season (FAO, 2021). Smoked fish increases protein availability all year round and easy fish packing and transportation to markets.

By far, fish salting, sun drying, fermenting or smoking are the traditional techniques employed in preservation in Ghana but mostly, the combination of salting with fermentation seems to be effective (Plahar *et al*., 1991; Sakyi *et al*., 2019). Ten to fifteen per cent of fish landed in Ghana are preserved by straight sun drying. Anchovies, sardines, trigger, tilapia, and moonfish are examples of fish that are sun-dried (Plahar & Lu, 1989). Usually, the fish are washed and spread on the ground (beach sand or fine gravel) or on mats and allowed to dry for 3-5 days(Ghaly *et al*., 2010; Nwaigwe, 2017). However, fish processing in Ghana is confronted with a few challenges. Fish is subjected to microbial and chemical contaminants immediately after catch, then through processing, storage, and finally in the open local markets.

Fish Smoking in Ghana

Traditional fish smoking in Ghana is artisanal and is predominately practiced by women in coastal towns, villages near the lake Volta and river banks (Aheto *et al*., 2017; Plahar *et al*., 1991). This sector is significant in relation to employment, income generation, and foreign exchange revenues which assist in sustaining the economy of Ghana *(*Asiedu *et al.,* 2018; Sakyi *et al*., 2019). An estimated 80 percent of fish caught in Ghana are processed traditionally (Adu-Gyamfi, 2006). Fish smoking procedure require basic equipment such as smoking ovens or kilns, stainless table knife, wood as fuel. Water used in thawing and washing fresh fish before smoking are fetched from

public water pipe or from the closest wells, rivers or sea shores (Asiedu *et al*., 2018).

Traditional smoking ovens

Preceding the 1970s, the most used ovens for smoking fish in Ghana were constructed with mud or metal and were either cylindrical or rectangular in shape (FAO, 2021). However, these ovens had several drawbacks, including excessive touching of the fish during the smouldering process, which particularly was problematic with smaller species like anchovies. Additionally, these ovens had low capacities, used fuel inefficiently, and were unable to handle the large quantities of fresh fish caught during bumper fishing periods invariably translates into high post-harvest losses (Sakyi *et al*., 2019). Consequently, an enhanced traditional smoking oven, known as the chorkor, was developed and presented in 1969 because of the constraints and disadvantages associated with earlier ovens. This new version was created by the Food and Agriculture Organization of the United Nations (FAO) and the Food Research Institute of the Council of Scientific and Industrial Research (CSIR) in Ghana.

The Chorkor has exhibited the capability of traditional knowledge in solving modern challenges. The chorkor kiln is a type of fish smoker named after a small fishing village located in the Accra metropolitan area, Greater Accra region. It offers numerous advantages such as small cost of production and using locally available materials to assemble. Plus, its huge capacity, moderate firewood consumption, and quick smoking time, this simple technology produces quality smoked fish. The popularity of this method in Ghana can be attributed to the participatory approach used to promote it,

which involved fish processors from the onset, ensuring high acceptance and adoption rates (Hasselberg *et al*., 2020; Sakyi *et al*., 2015). However, fat and moisture from the fish dripping into open flames create harmful compounds such as polycyclic aromatic hydrocarbons (PAHS) that have carcinogenic and mutagenic properties (Hasselberg *et al*., 2020).

Traditional fish smoking involves the direct burning of wood to produce heat and smoke (FAO/WHO, 2018). Fish smoking generally involves two stages, cooking and smoking stage, depending on the fish type, size and storage period, the fish either smoked soft or hard. During the cooking, heat is required, hence the fire is stroked with more firewood. However, fire intensity is controlled by the fish processor, where wood (fuel) is added into the smoker to increase fire intensity and vice-versa to reduce fire intensity. With regards to temperature, traditional fish smoking is of two methods (Baten *et al*., 2020). The smoking process is either "wet" or "dry" hot smoked where both procedures are performed at temperatures exceeding 80 °C, adequate to smoke the fish but the duration and final moisture content of the products differs. Hence, the fish type, size, and storage period determine the appropriate method to employ. Wet smoking takes 1-2 hours, yielding a product of about 40 to 60 percent moisture content, which is soft with a noticeable smoky aroma but cannot be stored for a long time (Belichovska *et al*., 2019), dry hot smoking on the other hand takes 10-18 hours and yields products with low moisture contents 10-15% (Hasselberg *et al*., 2020; Rumape *et al*., 2022).

Hot smoking process

Hot smoking procedure is employed to fish samples that are for immediate consumption or to be kept for 24-72 hours (Nwaigwe, 2017). On

receipt of fish stock, the fish are initially grouped according to weight and if frozen, they are defrosted in the open air or with water in lager basins. Fish thawing and washing are performed concurrently to remove slim, blood stains, and physical contaminants. Sometimes, defrosting can be performed in brine solution (3-4%) at 20-25℃ (Belichovska *et al*., 2019). Prewashed fish are loaded onto racks or trolleys and are allowed to air dry at the humid temperature range of 28℃ to 31℃ or on the smoking kiln for about 20 to 30 mins.

This stage intends to dry the outer parts of the fish since dry surfaces can absorb smoke and avert soot accumulation (Mezenova_O.YA., _Kim_I.N., 2001), providing an appealing appearance to the smoked fish skin (Akande *et al*., 2012). The loaded trays are transferred to the smoking kilns where smoking begins, as a final step in fish processing. This is performed at 80 to 120℃ for 30 minutes to 2 hours. Finished hot smoked fish is allowed to cool at ambient temperature, sorted by size and arranged in woven baskets or on wooden trays and transported to the nearest market to sell.

According to Hasselberg *et al*., (2020), 75% of fish landing in Ghana are processed traditionally. Smoked product quality depends on the artisanal skills and experience of the fish processor. A survey by Onumah *et al*., (2020) and Quaye, (2018) revealed that most Ghanaian households consume salmon, sardine, sea bream, and anchovy. Some traditional fish processors in Ghana operate in open or under sheds, which are dusty and without proper refuse disposal systems. This has become problematic as contamination can take place at all stages of processing and post-processing.

Food Hygiene

Seafood is rich in vitamin A, B, and D, iodine, selenium, calcium iron and long-chain omega-3 fatty acids (Elbashir *et al*., 2018). However, the quality and safety of fish and fishery products are of great concern globally, since it is a key contributor to food and nutrition security (Ryder *et al*., 2014). The consumption of smoke-flavoured fish can pose health risks due to biological and chemical contaminants, including pathogenic bacteria such as *Listeria monocytogenes*, *Clostridium botulinum*, histamine, and polycyclic aromatic hydrocarbons (PAHs) (Lin *et al*., 2008). Consuming smoke-flavoured fish may pose health risks due to biological and chemical contaminants, which include pathogenic bacteria like Listeria monocytogenes, Clostridium botulinum, histamine, and polycyclic aromatic hydrocarbons (PAHs), as stated by Lin et al. in (2008). Food safety ensures the consumers that the food is prepared, served, and consumed safely without causing any illness to them.

Biological hazards

Despite smoke's antimicrobial properties, certain pathogenic bacteria, such as *Listeria monocytogenes* and *Clostridium botulinum*, can still survive in fish products even after undergoing heat treatment (Novoslavskij *et al*., 2016; Sheng & Wang, 2021). *Listeria monocytogenes* is commonly found in nature and can infiltrate fish processing facilities through contaminated water sources, utensils, and cutting boards used during processing, ultimately contaminating the final products (Köse, 2010). According to FAO & WHO, (2020), smoked fish in particular carries the danger of listeriosis in cold smoked products compared to hot smoked product fish. Although there is limited evidence that *L. monocytogenes* in food at levels below $<10^2$ can cause

disease, the FDA's policy of zero tolerance for ready-to-eat products mandates the absence of *L. monocytogenes* in 25g of food samples (Lin *et al*., 2008).

Clostridium botulinum is anaerobic, motile rods that are commonly found in marine environments, gills, viscera of fish, crabs and shellfish (Lin *et al*., 2008). As reported by Amponsah *et al*. (2020), the occurrence of *Clostridium perfringens* in smoked fish can be ascribed to dumping sites and the dusty nature of the local markets where fish are sold. However, spores produced by *C. botulinum* can survive hot temperatures during fish smoking. Hot smoked-fish have a high risk of *C. botulinum* compared to cold-smoked fish, probably due to under-heating procedures that activate spore formation (Erkmen & Bozoglu, 2016). Sikorski & Kolodziejska (2002) have outlined procedures that involve a combination of temperature to damage the spores and salt treatment to inhibit the bacteria.

Heat stable organisms like Bacillus, Yeast and molds have been detected in hot smoked fish in Ghana (Sakyi *et al*., 2019). The quality of raw fish, handling, processing, and storage determines the microbial quality of the product produced. In Ghana, seventeen genera of microorganisms including food spoilage microbes have been identified from smoked anchovies and feed mills (Arthur & Osei-Somuah, 2004). Adu-Gyamfi, (2006); and Plahar *et al*., (1991) detected *coliform* spp., *bacillus* spp., *micrococcus* spp, *coryneform* group, *Klebsiella pneumonia*e, *Enterobacter* spp, *Proteus mirablilis*, *Serratia plymuthica*, *Erwinia* spp, *Aspergillus* spp., and *Rhizopus* spp., *Penicillium* spp., and yeasts. Obodai *et al*., (2011) detected *Salmonella* spp, *Staphylococcus aureus* and *Escherichia coli* in some selected smoked fish in

Tema, Ghana. Food hygiene indictors like *E.coli* and *Salmonella spp*. presence in food raises food safety concerns.

Biogenic amines and histamine

Food spoilage bacteria belonging to genera *Vibrio*, *Photobacterium*, *Klebsiella* spp. and *Morganella* spp. produce the enzyme histidine decarboxylase that converted histidine to histamine (Mayer & Fiechter, 2018; Ruiz-Capillas & Herrero, 2019). According to Ruiz-Capillas & Herrero (2019), smoking preserves the fish products by denaturing enzymes but histamine once already formed is not destroyed. Smoked fish products are known to contain elevated levels of histamine, which exceed the permitted levels set by the FDA or EU (Köse, 2010). Poor handling during packaging and storing can lead to recontamination and the production of histamine in final smoked products (Lin *et al*., 2008).

Chemical hazards

Smoke is a mixture of more than 300 chemicals that are released when wood is burned for smoking or during the process of drying fish. These chemicals include polycyclic aromatic hydrocarbons (PAHs), dioxins, formaldehyde, nitrogen, and sulfur oxides. Although these compounds can protect smoked foods from microorganisms and oxidative reactions, they may also contain harmful substances that can affect human health.

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and environmental contaminants that can be found in food (Costa, 2016; FAO, 2019). Contamination of foodstuffs by PAHs can occur at source through atmospheric gradual accumulation on crops, or preservation of food by drying and cooking methods. This contamination increases during intense heat

processing which gradually enter the inner fish flesh (Goulas & Kontominas, 2005). For several decades, smoked foods are recognised as the primary source of PAH particularly benzo(a)pyrene (Stołyhwo & Sikorski, 2005). During fish smoking, different smoke compounds, including aldehydes, ketones, alcohols, acids, hydrocarbons, esters, phenols, and ethers, are build up onto the fish surface. Since PAH have lipophilic properties, it can build up in the lipid portion of foods and are not readily removed from foods with elevated fat content (Kwaghvihi *et al*., 2020). The European Union (EU) has established strict regulations for smoked food products. The EU directive on smoked foods, Regulation No 2065/2003, sets limits on the content of BaP to be 10 µg kg-1 and benz(a)anthracene to be 20 µg kg-1 (Lin *et al*., 2008).

Factors Contributing to Microbial Growth in Smoked Fish

Most food, whether raw or processed, contains various species of microorganisms, including bacteria, yeasts, and filamentous molds (Rjeev *et al*., 2012). Seafood carries a variety of microorganisms from their natural environment. The behaviour of microbes in food depends on the chemical and physical composition of the food. Fish contains diverse nutritional compounds like carbon, nitrogen, vitamins, fats, and carbohydrates that support microbial growth. The survival and multiplication of any species or strain, whether desirable or not, are affected by the presence of other species. Factors such as extrinsic factors (pH, temperature, water, growth inhibitors) and environmental factors like atmospheric gases affect the response of microorganisms in food. The extrinsic factors concerning foodborne pathogens have been extensively studied (Anderson, 2018; ICMSF, 1980).

Moisture content

Drying is a traditional method of food preservation that involves removing moisture to prevent microorganism growth (Jay *et al*., 2005). Water activity (aw) refers to the amount of free water that is available for biological functions. The value of water activity ranges from 0 to 1, with 1 indicating the presence of free water in the food. This concept is closely related to the relative humidity (RH) of a food, which can be calculated as RH = 100 × aw (FDA, 2001). A_w of pure water is 1.00 and a completely dehydrated food is a_w of 0.00. The a_w of food is in the range of 0.10 – 0.99. Foods including vegetables, meat and fish have high a_w level of 0.98 while dry foods like cereal, salt sugar are between 0.1 – 0.20 (Erkmen & Bozoglu, 2005).

Bacteria require higher water activity (aw) values of about 0.91 to cause food spoilage, while moulds can do so at a lower aw of 0.80. Some food toxic bacteria like Staphylococcus aureus can grow at a minimum aw of 0.86, whereas Clostridium botulinum cannot grow below 0.94. The lowest reported aw value for foodborne bacteria is 0.75, which is suitable for halophiles (salt-loving). On the other hand, xerophile (dry-loving) moulds and osmophile (which prefer high osmotic pressures) yeasts have been reported to grow at even lower aw values of 0.65 and 0.61, respectively (Jay *et al*., 2005).

pH

The pH value for food varies significantly, food pH can be categorized as low to medium acidic (5.2 -6). Generally, microorganisms multiply at varying pH range of 6.6 - 7.5. (Jay *et al*., 2005). But at pH value below 4.0, yeasts grow better than bacteria, whereas, only moulds grow at pH values

below 1.5. Relatively low pH inhibits the growth of food-borne microbial pathogens including toxin-producing *S. aureus*.

Nutritional composition

Microorganisms require various essential nutrients for metabolism and growth. Additionally, the amount and type of nutrient required depends greatly on the microorganism. Almost all microorganisms use simple sugars like glucose, fructose, and maltose as sources of carbon and energy. A few special microbes produce special extracellular enzymes (amylase, lipases and proteases) which require starch and cellulose (complex sugars), nitrogen and lipids as energy and carbon source (Rjeev *et al*., 2012). Amino acids are nitrogen sources used by most microorganisms, but some microbes have enzymes that synthesizes proteins and nucleotides, to obtain nitrogen.

Fats are also used as energy but relatively small groups of microbes (moulds, yeasts, and few Gram-negative bacteria) require it (Erkmen & Bozoglu, 2005). Water is not a nutrient is however vital for biochemical activities in varying amounts, moulds require least amount followed by Gram negative bacteria, yeast and gram positive bacteria (Jay *et al*., 2005). Different foods have varying nutrients needed for microbial growth (FDA., 2011).

Fish and meat are rich in proteins, minerals, lipids and vitamins but poor in carbohydrates, plants based foods are generous in carbohydrates and dietary fibre but relatively poor in proteins, minerals, and some vitamins (Rjeev *et al*., 2012; Sandulachi, 2016). Bacteria species particularly, Gram negative rods including *Pseudomonas*, *Acinetobacter* spp., *Moraxella* spp., *Shewanella* spp., and *Aeromonas* spp., as well as pathogenic *Clostridium botulinum*, are proteolytic and grow well in protein-rich foods (meat and fish)

(Rjeev *et al*., 2012). Yeast and mould ferment carbohydrate-rich foods while some bacteria species of the genera *Bacillus* spp., *Clostridium* spp., *Aeromonas* spp., *Pseudomonas* spp., *Leuconostoc* spp., and *Enterobacter* spp. are saccharolytic that breakdown carbohydrates (Banwart, 1989).

Storage temperature

Reducing the temperature (-4 to -20℃) decreases the rate food of decay. Storage temperature is among the many critical factors that encourage microbial growth in food resulting in spoilage. The action of enzymes involved in microbial growth doubles at every 10℃ rise in temperature (Knipe & Rust, 2010). Conversely, with a decrease in temperature by 10℃, the rate of enzyme reaction is reduced by half. The FDA, (2011) recommends a temperature -20℃ for long-term storage for food and 4℃ temperatures for short-term storage in the refrigerator.

Food Safety Approach

The safety food is crucial in human health since consumption of unsafe and contaminated foods can lead to serious foodborne disease outbreak (UNIDO, 2020). The World Health Organization (WHO) reported in 2015 that contaminated food products cause illness in 1 out of every 10 people. Over 200 identified illnesses are transmitted as a result of food consumption (Kamboj et al., 2020). Each year, around 600 million people fall sick from consuming unfit food, leading to the loss of 33 million healthy life years. Children under five are the most affected, with over 125,000 deaths attributed to foodborne illnesses (Cole, 2004). Food supply chains have become increasingly complex, leading to longer and more frequent transportation of food and agri-food products.

This complexity increases the chances of contamination, as food can become contaminated during the transportation from slaughtering or harvesting, processing, to storage (F A O Food nutrition, 2006). This makes it challenging to track and identify the source of foodborne illnesses, because it can negatively affect the health of consumers in different regions (UNIDO, 2020). Unfortunately, the risk of contamination is higher for low socio-economic groups as a result of poor environmental surroundings, personal hygiene, water quality, unhygienic handling, inadequate heat treatment, and storage (WHO, 2006). According to the WHO, (2006), contaminated food or water causes nausea, vomiting, abdominal pain, and diarrheal diseases, resulting in 1.8 million deaths annually.

Further, WHO recognizes the crucial role of food handlers in ensuring the safety of food for the consumers. In the early 1990s, WHO developed the ten golden rules for safe food preparation, which were widely translated and disseminated. However, it later became clear that a more concise and universally applicable set of guidelines was needed. After almost a year of discussion with food safety experts and risk accessors, WHO introduced the five keys to safer food poster in 2001. These guidelines emphasize five essential practices for ensuring food safety: keeping things clean, separating raw and cooked foods, cooking thoroughly, keeping food at safe temperatures, and using safe water and raw materials (WHO, 2006).

Moreso, safety of food products like fish, poultry and meat products have been controlled by the inspection of finished product (Hajmeer, 1996). A subcommittee in 1971, at a national conference presentation on food protection, provided specific and critical approach to control microbiological

hazards in food than the former intent of inspection of the final products. Hazard Analysis Critical Control Points (HACCPs) was directed towards detecting food safety hazard upstream during production or manufacturing rather than in the finished products.

GMP/HACCP and Codex Alimentarius are systematic approaches based on science and technology, aimed at ensuring the safety of production and processing (FAO/WHO, 2018; Hajmeer, 1996). Principles of HACCPs is focused on food safety, not quality as indicated by the International Standard Organization 9000 series system (ISO9000). The ISO9000 system aims to provide a common standard quality during production or manufacturing of products, assuring two or more trading partners (locally, internationally) agree on the quality of the product. The regulations cover the testing of products, manufacturing, storage, handling, and distribution to ensure that the overall practices provide quality and safe products that are hazard-free. The system consists of (a) identification and assessment of hazards associated with growing, harvesting of raw material, processing, market preparing, and food products; (b) determining critical points to control of identified hazards; (c) establishing systems to monitor critical control points; finally monitoring through microbiological testing, physical and chemical tests as well as visual observations (Anderson, 2018; Ray & Bhunia, 2013).

Hence, food manufacturers and production industries must adhere and implement Good Manufacturing Practices (GMPs) to ensure all their products are manufactured in safe and healthy surroundings. This guarantees that the safety and quality of their products meet the standard requirements as set by Hajmeer (1996). Hence, assures consumer confidence that proper testing,

safety and quality checks have been maintained all through the manufacturing, packaging, and distribution of products. Ensuring the safety, quality, availability, affordability, and nutritional value of food to the consumer's heal

CHAPTER THREE

MATERIALS AND METHODS

Study Area

The study was conducted on two small to medium-scale fish smoking facilities that were in active production and used the chorkor smoking oven, which is Ghana's most frequently used smoking oven (Sakyi *et al*., 2019). These facilities were located (Figure 1) in the Duakro (GPS, CC-195-0961) and Brofoyedru (GPS, CC-024-0046) suburbs of Cape Coast, a metropolitan city situated in the central region of Ghana (latitude 5.1053, longitude -1.2466). Cape Coast covers a land area of 124km2, representing approximately 4.1 percent of the over-all land area of Ghana (Sonne *et al*., 2013). Cape Coast Metropolis, as the capital of Central Region of Ghana has a total population of 2,859,821, out of which 1,390,987 are males and 1,468,834 are females (Statistical Service Ghana, 2021).

Experimental Design

This research was conducted in two stages with two small-scaled fish processors in Cape Coast. The availability and popularity of frozen mackerel among the diverse socio-economic groups in Ghana contributed to the selection of mackerel in this study. Adopting the purposive sampling method, the first phase involved the evaluation of the general working conditions of the two fish smoking facilities through visual observation and random collection of samples at each processing step for microbiological and physicochemical analysis. During the second phase, corrective measures were implemented to ensure compliance with the Codex Alimentarius logic sequence guideline (Alimentarius, 1979) on good manufacturing practices. To assess the

effectiveness of applying a basic GMP/HACCP procedure, fish samples were randomly selected and analysed for quality again.

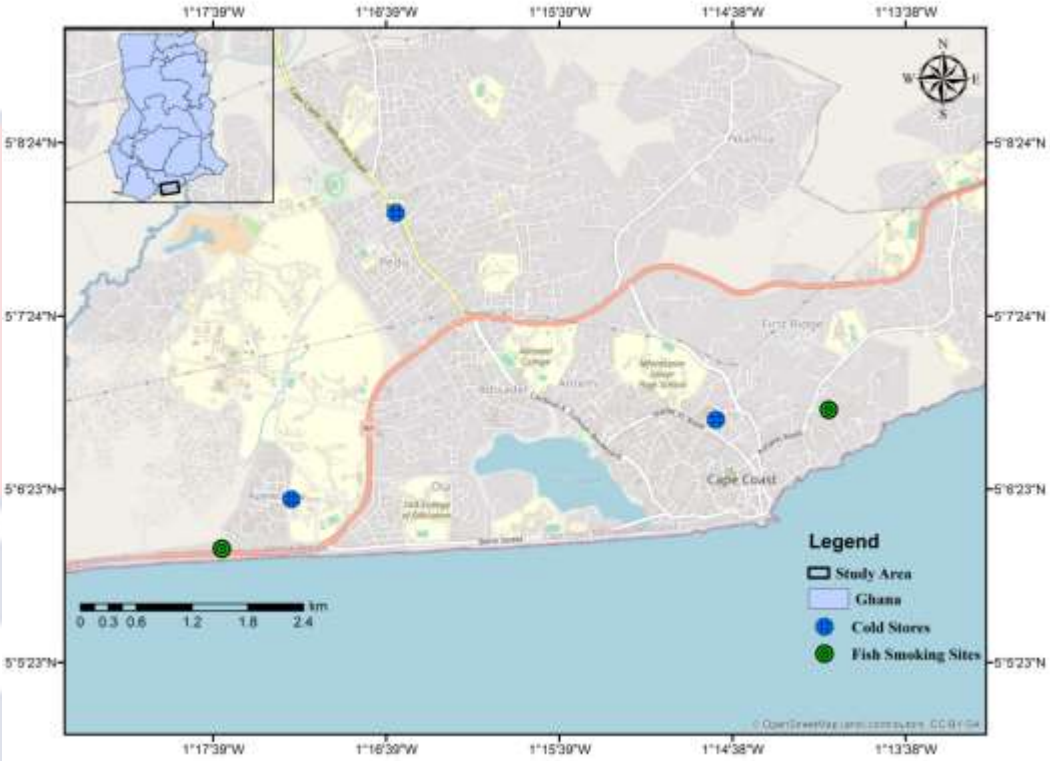

Figure 1: Geographic location of cold stores and smoking sites where samples were obtained for the study.

Fish Smoking Process

To assess the effect of GMP/HACCP application on the quality of smoked fish, the processors were allowed to apply their pre-existing knowledge for processing smoked fish without any interventions. The research team understudied the entire process chain from the raw material (unsmoked fish) acquisition stage to the packaging stage of the smoked fish. This was done so as to allow for the documentation of the fish smoking process (Figure 1).

Chub mackerel (Scomber japonicus) and the Chorkor oven are the two most commonly used fish and smoking method respectively by fish smokers in Ghana. For the study, we selected these two types of fish and smoking method. The first phase of fish smoking was carried out on March 18th and March 25th, 2022, at Duakro and Brofoyedru sites respectively. Subsequently, similarities and differences in the smoking process of the two processors were noted and harmonized to obtain a single fish smoking methodology in which the basic GMP/HACCP system was applied. Then the second phase of smoking was conducted on 15th and 22nd June 2022 at processing sites 1 and 2, respectively. Fish samples were transported in sterile polythene bags to the Laboratory of the Department of Biochemistry, University of Cape Coast and kept in a refrigerator at 4-6 °C until analyses.

Fish Quality Analyses

Microbiological and physicochemical analyses were carried out on fish samples produced before and after implementing the basic GMP/HACCP to determine the impact on fish quality.

Microbiological Analyses

Sterilization procedure

All glassware, including Petri dishes, test tubes, and media preparation bottles were washed with detergent, rinsed under flowing tap water, and oven-dried at 60℃ to remove water residue. Heat-stable translucent polythene bags used for sample collections were folded and individually wrapped in aluminium. Petri dishes wrapped in brown paper, aluminium-wrapped polyethene bags together with pipette tips and all other glassware were sterilized in an autoclaved for 15 minutes at a temperature of 121℃ and

similarly oven dried. Forceps and knife were flamed with spirit lamp until hot red, cooled briefly in the air before and after each usage. The entire surface of the bio safety cabinet was sterilized with 70% ethanol before and after each use.

Culture Media Preparation

Plate count agar

Plate Count Agar (PCA, Oxoid code CM 0325, England), 17.5g/L, Violet Red Bile Glucose agar (VRBG, Oxoid code CM 0485, England), 38.5 g/L, Eosin Methylene Blue agar (EMB, Oxoid code 0069, England), Sabouraud Dextrose Agar (SDA, Oxoid code CM 0041, England), 65g/L were prepared separately by transferring required amount into a beaker containing one litre of distilled water. The mixture swirled to disperse the media, heated in the microwave for 3 minutes and transferred into media bottles and corked screwed with its cover and sterilized in an autoclaved for 15 minutes at 121 °C.

Preparation of phosphate buffered saline

About 800 ml of distilled water was added to a beaker, followed by 8 g of sodium chloride and potassium chloride (0.2 g). Then, 1.44g of sodium phosphate dibasic and 0.245g of potassium phosphate monobasic were added. The solution was stirred to dissolve the reagents completely and pH adjusted to 7.2. The solution was transferred into one litre volumetric flask and made up to mark with distilled water (Pitt & Hocking, 1997).

Sample Collection

Sampling according to ICMSF, (1980) was used for microbial analysis. Three pieces of fish were sampled randomly at each processing stage (raw,

washed, drained, and smoked), placed on ice packs and transported to the laboratory. Samples were stored at a refrigerator temperature of 4℃ for 24 hours before the microbial analysis was conducted. Measurements of physical qualities like texture, colour, length, and pH were taken on arrival at the laboratory. Samples for chemical analysis were also stored in the freezer at -20 ℃ until analysis. From each processor, samples were taken twice before and after GMP/HACCP implementation for analysis.

Sample processing before microbial analysis

Under aseptic conditions in the safety cabinet (BSC-IIA2-AJ), fish were randomly sampled and placed on a tray. With the aid of a sterile knife, 15 g of dorsal, ventral and posterior fish flesh was cut and homogenized in a blender with 135 ml of sterile phosphate buffer saline (PBS) for 30 to 60 seconds to prepare fish homogenate (Alpha, 2012). Microbial contaminants were enumerated as follows.

A volume of 1 ml of the fish homogenate was transferred to a test tube containing 9 ml sterile Phosphate Buffered Saline (PBS) to prepare a 10-fold serial dilution. Using the pour-plate technique, 1 ml of each of the diluted samples was inoculated into sterilized Petri dishes and molten PCA, VRBG agar, and EMB agar, precooled to 45 ℃ was added. The Petri dish was covered and mixed gently by swirling agar plates clockwise, and anticlockwise to ensure a uniform distribution of samples in the medium. The inoculated agar plates were allowed to solidify and were subsequently incubated aerobically at 37℃ for 24 hours in a MIR 154 Fisher scientific cooled incubator.

The total fungal counts were similarly determined using the pour-plate technique, using Sabouraud Dextrose Agar supplemented with chloramphenicol (1 mg/ml) as described by Zafar *et al*. (2017). Petri dishes were covered and thoroughly mixed after which the inoculated agar plates were allowed to solidify and incubated at room temperature (25 ± 2 ℃) for 3-5 days.

At the end of the incubation, culture plates were examined and plates containing colonies ranging from 30-300 were counted for bacteria and culture plates containing 10-150 colonies were counted for fungi using Reichert Colony Counter (US, Technologies). The Original Cell Density (OCD) of total bacterial and fungal per gram of fish sample was calculated using the formula:

$$OCD = CFU/(D \times V) \quad 1$$

where:

OCD = Original Cell Density, CFU= Colony Forming Unit counted on agar plate, D = Dilution factor of inoculum, V = Volume of inoculum plated.

The total bacterial and fungal counts of each sample was calculated from the average of the triplicate values of total bacterial or fungal load obtained (Reynolds, 2011).

Physicochemical Analysis

pH value

pH was measured by weighing 10 g of each sample (raw and smoked) homogenized with 50 ml distilled water for 30s. A pH meter (Inolab pH730) equipped with a glass electrode was used to determine the pH values (Khodanazary, 2019). Duplicate determination was done in all cases.

Colour measurement

Colour was determined according to procedure by Cardinal *et al*., (2001) using CHN SPEC CS-10 colorimeter to take the measurement from the head, middle, and tail areas of the raw and smoked fish. Colours was expressed as L*, a*, b* according to international Commission of Illumination (CIE) lab coordinates.

Texture measurement

The GY-4 digital fruit penetrometer was used to measure the texture of fish muscle after removing skin from the back, belly, and tail regions. Texture measurement was taken as described by Casas *et al*. (2006).

Weight and length measurement

Total length measurement was recorded to the nearest millimetre on a straight meter board. From fish snout to the end of caudal fin as described by Önsoy *et al*.,(2011). Thawed and smoked fish samples were weighed using a kitchen scale (Ozeri ZK24), following the method by Baten *et al*. (2020).

Proximate Analysis

Sample preparation

The raw fish samples were eviscerated, washed to remove residual blood, and then filleted lengthwise along the backbone to obtain fish flesh excluding bones. The fillets were diced into pieces and dried in an oven (VWR DL 56 prime) at a temperature of 50℃ for six days. After drying, the fillets were ground into powder and transferred into a clean zip lock bag, labelled and kept in the freezer at -20 ℃ pending further laboratory analysis.

Determination of percentage (%) moisture content

Moisture content of the fish samples were determined using oven dry procedure (Reeb & Milato, 1999). By weighing five grams (5 g) each of sample into crucibles of known weight. Crucibles containing samples were placed in a preheated oven at a temperature of 105℃ for 3 hours. Samples were taken out from oven and allowed to cool in desiccators before re-weighing and placed back into oven for further drying. This procedure of drying, cooling, and re-weighing continued till a constant weight was attained. Percentage moisture was calculated with the formula in equation 2:

$$\%\ Moisture\ content = \frac{Initial\ weight - Oven\ dry\ weight}{Oven\ dry\ weight} \times 100 \quad (2)$$

Lipid content

Lipid content was determined following the Soxhlet method previously described by (Noureddini & Byun, 2010) using a Soxhlet 2050 automated analyser. Petroleum ether (40-60% boiling point) was used for the extraction, where percentage of lipids was calculated following the equation below:

$$\%\ lipid = \frac{Weight_{(extraction\ cup\ +residue)} - Weight_{(extraction\ cup)}}{Weight_{sample}} \times 100 \quad (3)$$

Determination of ash content

Procedure described by Feng *et al.* (2005) was adapted to determine the Ash content of the fish samples. By weighing five grams (5 g) of each sample into a crucible of known weight. The crucible with samples was placed in a preheated furnace set at a temperature of 550℃ for 6 hours. The ash residue was taken from the furnace and allowed to cool before weighing again.

The heating, cooling and re-weighing process continued until a constant weight was obtained. Percentage ash content was determined using the equation:

$$\% \, ASH = \frac{(Wt.\ crucible\ and\ ash - \ crucible\ Wt.}{(Wt.\ crucible\ and\ sample - \ crucible\ Wt)} \times 100 \quad (4)$$

Determination of crude protein

The modified method described by Mæhre *et al.*, (2018), was adapted to quantify protein content in fish samples. One gram (1g) of the sample was weighed into micro-Kjeldahl digestion flasks, in addition, 7g of digestion catalyst, 2-3 pumice beads (bump prevention) and 20ml of concentrated sulphuric acid were added. The flask was inclined and heated gently until foam disappeared, then boiled for 2 hours until light green clear solution was obtained. The solution was cooled by gradually adding distilled water and was transferred into a 100mL standard flask and made to the volume with distilled water. Blank was prepared with reagents without sample. The diluted digest was transferred into a micro-kjedahl distillation apparatus (reaction chamber) and rinsed with distilled water. Additionally, 40% sodium hydroxide and two drops of phenolphthalein indicator were added until solution turned pink. The receiver end of distillation unit was dipped in boric acid solution containing a drop of Tashiro indicator. The ammonia released was absorbed into a solution of boric acid and titrated with standard 0.1 N hydrochloric acid until the solution turned pink. Protein constituent in the fish samples was calculated by:

$$Nitrogen\left(mg\frac{N}{100g}\right) = 14 \times (b-a) \times N \times V_1 \times \frac{100}{V_2 \times W} = ''X'' \quad (5)$$

Where:

b = volume of hydrochloric acid used in sample titration

a = volume of sulphuric acid used in reagent blank titration

N = corrected normality of the hydrochloric acid for titration

V_1 = volume of the digested solution made up

V_2 = distilled digested solution volume taken for distillation

W = weight of sample

14= Nitrogen atomic number

$$Protein\ content\ (\%) = \frac{X \times 6.25}{1000} \quad (6)$$

Where:

6.25= nitrogen protein conversion factor for fish and fish products

1000 = factor to convert milligram to gram

Carbohydrate content

Carbohydrate content in fish samples was determined using the difference method and the following equation.

$$Total\ CHO = 10 - (moisture + ash + protein + total\ lipids)\% \quad (7)$$

Peroxide Value (PV)

Fish oil was extracted by adapting Soxhlet method previously described by (Noureddini & Byun, 2010). Five grams of fish oil was weighed into 250 ml of conical flask, then 30 ml acetic acid chloroform solvent mixture (3:2) was added and swirled to dissolve the oil. Exactly 0.5 ml of saturated potassium iodide solution was added to the sample in a conical flask. The resultant solution was kept in the dark for one minute with intermittent mixing

after which 30mL of distilled water was added and mixed intermittently, and then 0.5 mL of starch solution was added. The mixture was titrated against 0.01 N sodium thiosulphate with vigorous shaking to liberate iodine (AOAC, 2000). Blank value was determined without the oil. Peroxide value was expressed as milliequivalent of peroxide oxygen per kg sample (meq/kg):

$$peroxide\ value = \frac{Titre \times N \times 1000}{Weight\ of\ sample} \quad (8)$$

Where;

Titre = volume of Sodium Thiosulphate used (blank corrected)

N = Normality of sodium thiosulphate solution

Histamine Content

The method described according to Stroka *et al*., (2014) was used to determine histamine levels in the fish samples. Five grams (5g) of each sample was extracted after adding 100µl of 1,3-diaminopropane solution with 10 ml of 0.2 M perchloric acid and was homogenized for 1min in a blender (MX 703A). The extract was then centrifuged at 12000 g at 4℃ for 5min and 100 µl of the supernatant was pipetted into a glass vial after which 200 µl of 6.9M sodium carbonate solution and 400 µl of 10mg/ml dansylchoride solution was also added. The vial was closed, vortex and incubated for 5 minutes at 60℃ in the dark. Centrifuge tubes were cool at room temperature and 100 µl of 100 mg/ml proline solution was added. The mixture was vortexed again and incubated in the dark for 15 minutes. After incubation, 500 µl of toluene (99%) was added to the mixture and vortex and stored in the freezer at -18℃ for 30min. After freezing, the non-frozen, organic phase was collected into a new vial and evaporated at room temperature under nitrogen. The evaporated residue was redissolved in 200µl acetonitrile (99%) and was vortex at 12000

g. This mixture was filtered through an inline syringe filter and injected to 10 µl into the HPLC and detection was done at 254 nm.

Polycyclic Aromatic Hydrocarbons level

Polycyclic Aromatic Hydrocarbons (PAH) quantity in the smoked fish was determined as described by Smith & Lynam, (2012). Three grams (3g) of each of the fish was weighed into centrifuge tubes. An appropriate amount of PAH solution and QC solutions was added and vortexed for 1 minute. Then, 12 ml of deionized water and 15 ml of acetonitrile were added to the tube. Two ceramic bars (p/n 5982-9313) were also added and vortexed for 1 minute to extract the sample. An original Agilent Bond Elut QuEChERS extraction salt packet (p/n5982-6555) containing 6 grams of $MgSO_4$ and 15g of sodium chloride was added to each test tube. The tubes were pulverised for 1 minute at 1500 rpm. The samples were then centrifuged at 4000 rpm for 5 minutes. After which, 8ml of the upper layer was transferred to an Agilent Bond Elut QuEChERS fatty sample dispersive SPE 15 ml tube (p/n5982-5158). The dSPE tube was then vortexed for 1 minute and centrifuged at 4000rpm for 5 minutes to complete the sample extraction. The liquid phase from the dSPE tube was transferred to a GC vial and analysed by Agilent 5975B GC/MS.

Statistical Analysis

The data was recorded in Microsoft Excel, and then analyzed using the analysis of variance tool (ANOVA) to compare the effects of basic GMP/HACCP application on both the microbial and physicochemical quality of smoked fish. SPSS (IBM, SPSS Statistics V25.0) was used for this analysis. Any significance statement refers to a p-value of less than 0.05.

CHAPTER FOUR

RESULTS

The Fish Smoking Process before the Implementation of the GMP/HACCP Procedure

Before the basic GMP/HACCP was implemented, frozen fish (chub mackerel, *Scomber japonicus*), which was packed in 20 kg blocks, wrapped in a polyethene film and stored in paper boxes at -20 °C was procured from a local distributor at Abura market in Cape Coast, Ghana (GPS: CC-094-7899). The frozen samples were processed according to the flow chart in Figure 1 by transporting the fish to the two fish processing sites, allowing to thaw to room temperature and washing with tap water to eliminate blood, slime, and foreign materials.

The fish was racked to decrease the moisture levels either in the sun by Processor 1 or over a smouldering oven by Processor 2. Thereafter, the low-moisture fish was transferred into the locally constructed Chorkor oven and hot-smoked for 2 hours until sufficient cooking was achieved. Smoked fish was left to cool to room temperature overnight and packed in brown paper bags. Hot smoking is a preferred method in fish smoking in Ghana because the smoking intensity can easily be modulated by adjusting the quantity of firewood to achieve optimal fish cooking weight, moisture, colour and aroma as desired (Asiedu *et al*. 2018; Sakyi *et al*. 2019).

The choice of fish for smoking is generally based on its availability. Freshly harvested fish is preferred over frozen fish due to its low cost, especially during periods of high production. However, during the low season,

smokers have to rely on frozen fish from cold storage facilities (Asiedu *et al*., 2018; Sakyi *et al*., 2019).

As part of the evaluation fish smoking process, a flow diagram was created (Figure 2) to illustrate the traditional operations involved.

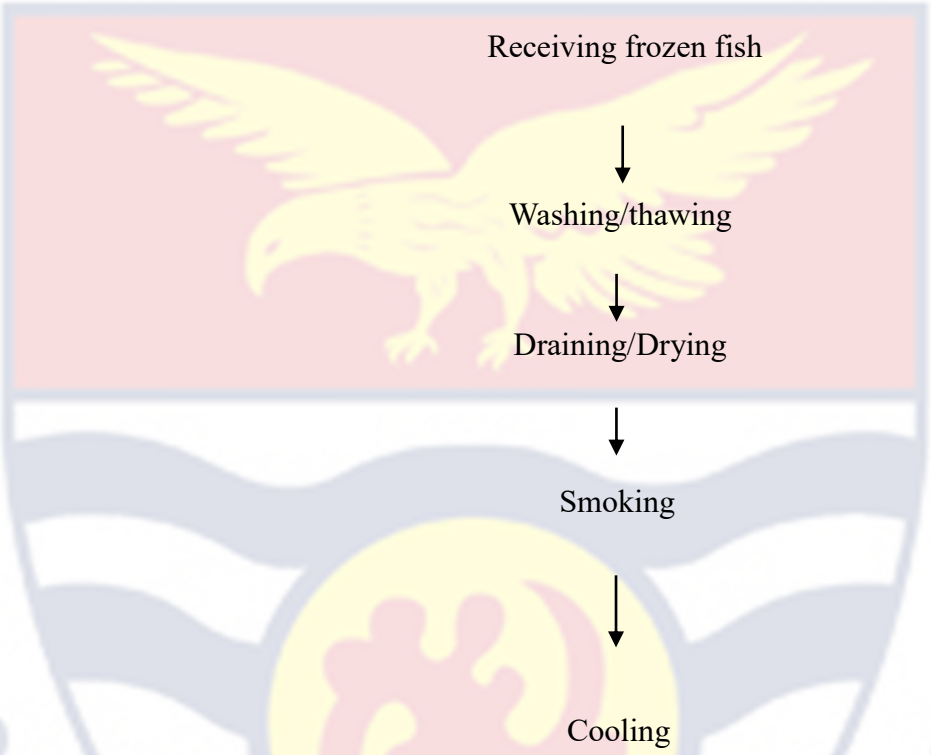

Figure 2. Steps involved in traditional fish processing

The identified critical control points were also included in the diagram to enhance the safety of smoked fish, based on Codex Alimentarius logic sequence (Table 1).

Types of Ovens Used in this Study

According to observations, processor 1 used a rectangular-shaped Chorkor oven with metal racks fitted in rectangular wooden molds that fit into the smoker. Meanwhile, processor 2 used a traditional round Chorkor oven with fresh neem sticks stacked inside (Figure 3).

Figure 3: The types of 'Chorkor' smoker
A: Rectangular shaped smoker
B: Round shaped smoker

Table 1: Hazard analyses of traditional fish processing based on the codex alimentarius logic sequence

Processing step	Potential hazard identified	Any potential food safety (Yes/No)	Justify your decision in column 3	Is this step a critical control point (Yes/No)	What control limit can be applied to prevent the significant hazard(s)
Transportation of frozen fish	Biological	Yes	Temperature abuse can increase microbial growth	Yes	Transportation of fish in a cold insulated container. To main the cold chain
	Chemical	Yes	Histamine may occur due to temperature abuse	Yes	Maintain a cold chain during transportation
	Physical	No	-	No	-
Receiving of fish	Biological	Yes	Microbial growth may increase with temperature abuse	Yes	Freeze or refrigerator storage, if fish is not going to be processed immediately
	Chemical	Yes	Histamine may occur due to temperature abuse	Yes	Freeze or refrigerator storage, if not going to be processed immediately
	Physical	No	-	No	-
Washing	Biological	Yes	Bacterial contamination from the water source	Yes	Potable water source and changing washing water often (after two washing)
	Chemical	Yes	Chemical and pesticide residue	Yes	Water must be from a potable source

Table 1: Cont'd, Hazard analyses of traditional fish processing based on the codex alimentarius logic sequence

	Physical	Yes	Weeds, sand particles from the water source	Yes	Water must be from a potable source
Drying	Biological	Yes	Prolong open atmosphere drying can lead to microbe multiplication	Yes	Dry on the stove, using smoked generated from the smoking kiln.
	Chemical	No	No	No	No
	Physical	Yes	Contamination from the environment (dust and sand)	Yes	Dry on the stove, using smoke generated from the smoking kiln
Smoking	Biological	Yes	Inadequate cooking will not kill all microbes	Yes	Proper smoking/cooking (80-120℃, 2-3 hours smoking/cooling time) reducing water activity to 0.98
	Chemical	Yes	Polycyclic Aromatic Hydrocarbons	Yes	Reduce excessive smoke by allowing good air circulation in the smoking kiln
	Physical	No	-	No	No
Cooling	Biological	No	-	No	-
	Chemical	No	-	No	-
	Physical	No	-	No	-
Handling and packaging	Biological	Yes	Recontamination with microbes	Yes	Observe Sanitary Standard Operation Procedures
	Chemical	No	-	No	-
	Physical	No	-	No	-

Implementation of the Basic GMP/HACCP

After conducting the gap analysis, interventions listed in Table 2 were implemented to meet the basic requirements for Good Manufacturing Practices (GMP) for hazard analysis of traditional fish processing. The Codex Alimentarius logic sequence in Table 1 was used to identify biological hazards as the main risk. Temperature abuse could lead to bacterial proliferation and traditional processors operate in open and dusty environments, which could predispose raw fish to blowfly infestations during the drying process. Blowflies lay eggs on fish, which enter the fish's internal parts and hatch into maggots making it unsafe for consumption.

Access to potable water is limited for most traditional fish processors, which results in the use of water that has been used multiple times. As a result, the processing water accumulates dirt, organic matter, and microbes, leading to cross-contamination among different batches of the processing unit (Bedane *et al*., 2022; Kirby *et al*., 2003). Figure 4 depicts the critical control points (CCPs) such as conditions for receiving frozen fish, freezer storage, washing/thawing, drying, smoking, and cooling in the traditional fish smoking process.

Table 2. Limitations and interventions implemented for the success of a basic GMP/HACCP procedure at the two smoked fish processing facilities

	Observation	Intervention
1.	Frozen fish were transported to the processing centre without any temperature control, which may have caused quality issues	During transportation, frozen fish were placed in ice chests along with ice cubes to ensure that the temperature was maintained
2.	No waste disposal unit was available at the processing centre	Bins for waste disposal were provided
3.	Some unit operations, eg. thawing/washing in bowls were done on the bare ground	Working benches served as platforms for bowls during thawing/washing of fish
4.	Thaw drips seeped onto the floor, which attracted flies	Thawing was carried out in containers and drips properly disposed.
5.	Fish draining prior to smoking was carried out in the open air	Nets were provided to cover fish during draining to keep away insects/flies
6.	Smoked fish was left in the open during overnight cooling	Nets were provided to cover smoked fish to keep away insects, rodents, and other pests
7.	Safety apparels were not used during fish processing	Hand gloves, hair covers, and safety coats were provided to all processors

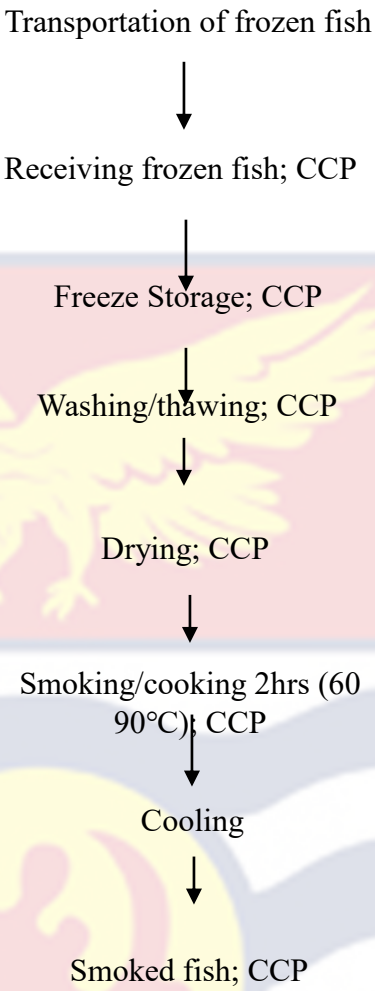

Figure 4: Traditional fish processing steps showing the critical control points (CCPs)

Microbiological Quality during Fish Processing

The total viable bacterial count (TVBC) of thawed fish samples was higher compared to the total fungal and coliform counts, while the *E. coli* count was the least. Table 3 displays the microbial counts (Log CFU/ g) of thawed fish for processor 1 (P1) and processor 2 (P2) for total viable count, coliforms, *E. coli*, and fungi, respectively. After the washing and draining step, there was a reduction in microbial load for TVBC, coliforms, *E. coli*, and TFC for both processors P1 and P2.

After smoking the fish and storing it overnight, there was no significant ($P < 0.05$) reduction in the total viable counts (Log CFU/ g) for P1 and P2. However, both processors showed a reduction in coliforms (40% and 36%) while P1 demonstrated a 27% reduction in E. coli count and P2 showed no reduction. Fungi, as the major microflora of smoked fish, had a 45% and 34% reduction in count for both processors (P1, P2) respectively. After applying the HACCP procedures, there was a significant reduction in TVBC counts for both P1 and P2, with a reduction of 39% and 28%, respectively, after washing and draining (as shown in Table 3). Additionally, smoking the fish resulted in a 100% reduction in bacterial counts (as illustrated in Figure 5), indicating that the counts were below the detection levels of less than 1.0 Log cfu/g. It's worth noting that while lower TVBCs were observed in the initial microbial loads of thawed fish, the HACCP procedure was still effective in reducing bacterial counts.

Table 3: Total viable, coliforms, *E. coli* and fungal count for fish processing stages

	Mean microbial count of fish obtained from 2 fishmongers before and after GMP/HACCP implementation (Log CFU/g)			
	P1		**P2**	
Processing steps	**Before GMP/HACCP**	**After GMP/HACCP**	**Before GMP/HACCP**	**After GMP/HACCP**
	TVC			**TVC**
Raw	4.18±0.02^{a}	2.49±0.08^{a}	4.20±0.13^{a}	2.57±0.04^{a}
Washed	3.60±0.03^{b}	2.04±0.06^{b}	3.98±0.03^{a}	2.14±0.09^{b}
Drained	3.20±0.08^{c}	2.08±0.00^{b}	3.95±0.04^{a}	1.98±0.03^{c}
Smoked	3.96±0.12^{d}	1.52±0.28^{c}	4.10±0.28^{a}	1.85±0.85^{abc}
	Coliforms			**Coliforms**
Raw	2.84±0.09^{a}	1.24±0.34^{a}	2.72±0.09^{a}	1.24±0.09^{a}
Washed	2.24±0.09^{b}	0.85±0.21^{a}	2.39±0.12^{b}	1.11±0.10^{a}
Drained	2.20±0.08^{c}	0.69±0.12^{a}	2.20±0.28^{b}	0.60±0.43^{a}
Smoked	1.69±0.12^{d}	0.00±0.00^{b}	1.74±0.37^{b}	0.00±0.00^{b}
	E.coli			**E.coli**
Raw	1.59±0.07^{a}	1.36±0.08^{a}	1.11±0.10^{a}	1.55±0.07^{a}
Washed	1.09±0.00^{b}	1.10±0.14^{a}	0.85±0.21^{a}	0.89±0.27^{b}
Drained	1.17±0.12^{b}	1.13±0.07^{b}	1.14±0.09^{a}	1.29±0.08^{a}
Smoked	1.15±0.21^{b}	0.00±0.00^{b}	1.24±0.37^{a}	0.00±0.00^{c}
	Fungi			**Fungi**
Raw	3.86±0.08^{a}	2.02±0.09^{a}	3.65±0.07^{a}	2.30±0.17^{a}
Washed	2.84±0.09^{b}	1.87±0.04^{a}	2.90±0.08^{b}	2.03±0.04^{b}
Drained	2.90±0.08^{b}	1.91±0.04^{a}	2.92±0.02^{b}	2.03±0.06^{b}
Smoked	2.09±0.12^{c}	0.45±0.07^{b}	2.39±0.12^{c}	0.35±0.28^{c}

Values are mean ± SD triplicate determination.

Mean values with different alphabet superscript before and after GMP/HACCP are significantly different at $p < 0.05$

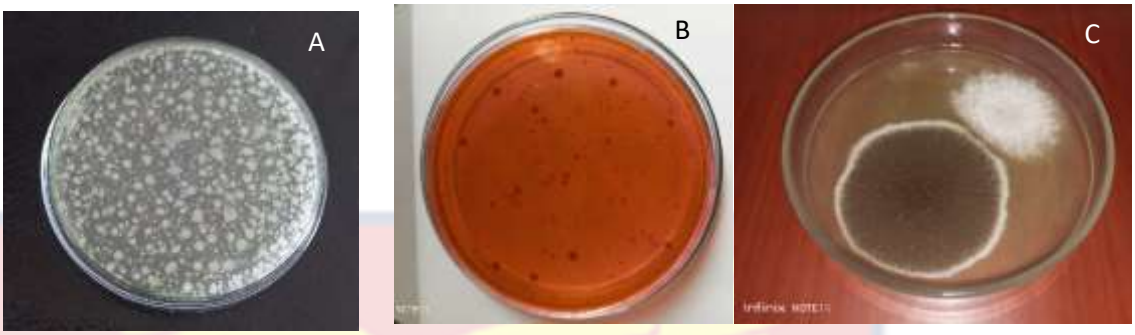

Figure 5: Mixed cultures of bacterial and fungal species

A: Bacterial cells on Nutrient Agar after 24 hours of incubation at 35 °C

B: Coliforms growing on Eosin Methylene Blue Agar after 24 hours of incubation at 35 °C

C: *Aspergillus spp* growing on Sabouraud Dextrose Agar at 5 days incubation at room temperature

Physicochemical Properties of Raw and Smoked fish

Table 4 displays the physical properties of fresh fish, which show no significant difference between weight, length (Figure 6), and hardness. However, smoking the fish resulted in significant browning, with a marginal decrease in weight and hardness. These properties were not significantly affected by the HACCP procedure. Before the implementation of GMP/HACCP, the browning index of smoked fish ranged from 4.53 to 17.28. However, after the application of GMP/HACCP, the browning intensity of the fish significantly increased to a range of 45.38 to 87.73. Furthermore, after GMP/HACCP implementation, the browning intensity of the fish increased even more, ranging from 117.94 to 181.43. The pH values of both fresh and

smoked samples also showed no significant change due to the smoking procedure (as indicated in Table 4).

Table 4: Physicochemical properties of frozen and smoked chub mackerel before and after GMP/HACCP

Parameters	P1				P2			
	Before GMP/HACCP		After GMP/HACCP		Before GMP/HACCP		After GMP/HACCP	
	Frozen	Smoked	Frozen	Smoked	Frozen	Smoked	Frozen	Smoked
Weight (g)	352.33±33.33^{a}	297.33±81.45^{a}	227.67±59.99^{a}	109.17±55.32^{a}	321.67±24.46^{a}	291.17±28.29^{a}	247.33±40.68^{a}	176.17±67.28^{a}
Length (cm)	34.00±2.37^{a}	31.33±1.97^{a}	30.17±2.40^{a}	25.67±3.01^{a}	33.67±3.01^{a}	33.83±2.99^{a}	30.33±0.82^{a}	28.83±2.14^{a}
pH	5.83±0.37^{a}	5.72±0.40^{a}	5.57±0.13^{a}	5.09±0.15^{a}	5.65±0.18^{a}	5.63±0.42^{a}	5.90±0.15^{a}	5.83±0.05^{a}
Brix	1.35±0.18^{a}	1.08±0.16^{a}	1.98±0.34^{a}	1.27±0.20^{a}	1.28±0.08^{a}	1.17±0.15^{a}	1.88±0.34^{a}	1.23±0.14^{a}
Texture	26.02±5.26^{a}	22.94±2.35^{a}	27.20±5.75^{a}	17.79±4.21^{a}	23.20±5.21^{a}	23.01±3.89^{a}	30.27±6.09^{a}	19.98±6.72^{a}
Colour L*	52.06±12.09^{a}	35.14±18.68^{a}	46.46±6.48^{a}	29.82±13.35^{a}	49.83±12.88^{a}	40.22±12.37^{a}	37.70±9.38^{a}	24.38±5.53^{a}
a*	2.96±10.09^{a}	7.23±4.74^{a}	7.95±4.34^{a}	23.66±15.81^{a}	0.07±6.20^{a}	6.06±6.39^{a}	13.89±9.31^{a}	33.99±8.57^{b}
b*	6.73±5.71^{a}	11.47±10.09^{a}	1.94±3.44^{a}	14.80±4,62^{b}	4.98±7.72^{a}	9.62±7.92^{a}	3.53±3.10^{a}	10.27±3.10^{b}
Peroxide value (meq/kg)	10.5±2.12^{a}	16.0±1.41^{b}	5.5±0.71^{a}	10.5±0.71^{b}	9.0±1.41^{a}	15.3±0.71^{b}	6.00±2.80^{a}	6.00±1.14^{a}
Histamine (mg/kg)	*	17.55 ± 5.90^{a}	*	5.62 ± 0.71^{b}	*	14.67 ± 1.92^{a}	*	3.85 ± 0.47^{b}

Values are means ± SD of triplicate determinations. Asterisk (*) means histamine analysis was not conducted
Mean values with different alphabet superscript before and after GMP/HACCP are significantly different at $p < 0.05$

Figure 6: Thawed frozen chub mackerel on measuring board.

Peroxide analysis is an important measure used to determine the level of primary oxidation in oils and oily foods. In this study, the average peroxide value of smoked chub mackerel (as shown in Table 4) was found to have significantly increased from 60 to 100% for both processors after implementation of GMP/HACCP. Additionally, the average histamine content reduced three-fold for both processors (P1 and P2) respectively.

Furthermore, after implementing GMP/HACCP, the levels of PAH4 in smoked fish (as shown in Table 5) were significantly reduced. Processor 1 experienced a reduction of 38% in Benzo(a)anthracene, 21.7% in Chrysene, 28.5% in Benzo(a)pyrene, and 36.9% in Benzo(b)fluoranthene. Processor 2 also saw a reduction in levels, with a decrease of 19% in Benzo(a)anthracene, 12% in Chrysene, 5% in Benzo(a)pyrene, and Benzo(b)fluoranthene.

Table 5: Effect of GMP/HACCP implementation on the PAH levels of smoked fish

PAH(µ/Kg)	Processor A		Processor B	
	Before GMP/HACCP	After GMP/HACCP	Before GMP/HACCP	After GMP/HACCP
Naphthalene	30.41 ± 3.32[a]	28.17 ± 2.54[a]	26.73 ± 3.72[a]	18.21 ± 1.59[b]
Acenaphthylene	64.78 ± 8.25[a]	43.45 ± 5.40[b]	59.54 ± 8.61[a]	34.16 ± 2.43[b]
Acenaphthene	3.18 ± 0.48[a]	16.67 ± 1.95[b]	3.87 ± 0.35[a]	15.06 ± 2.54[b]
Fluorene	42.69 ± 4.50[a]	27.76 ± 2.80[b]	40.31 ± 5.13[a]	21.29 ± 1.05[b]
Anthracene	43.19 ± 4.79[a]	31.96 ± 3.82[b]	31.82 ± 3.56[a]	31.05 ± 2.12[a]
Phenanthrene	89.05 ± 8.81[a]	68.06 ± 5.38[b]	80.84 ± 10.67[a]	72.96 ± 5.13[a]
Fluorathene	54.02 ± 6.14[a]	32.97 ± 3.33[b]	47.57 ± 4.98[a]	56.64 ± 3.27[b]
Pyrene	45.15 ± 3.94[a]	27.30 ± 2.64[b]	42.92 ± 4.84[a]	38.49 ± 3.01[a]
Benzo(a)anthracene	**24.25 ± 3.10[a]**	**15.01 ± 2.61[b]**	**23.37 ± 2.42[a]**	**18.87 ± 1.06[b]**
Chrysene	**38.04 ± 3.15[a]**	**29.76 ± 2.47[b]**	**38.53 ± 4.16a**	**33.83 ± 4.17[a]**
Benzo(a)pyrene	**20.13 ± 1.97[a]**	**14.38 ± 1.04[b]**	**20.26 ± 2.64[a]**	**19.24 ± 1.24[a]**
Benzo(b)fluoranthene	**25.64 ± 2.35[a]**	**16.16 ± 2.41[b]**	**24.76 ± 2.64[a]**	**21.34 ± 3.80[a]**
Benzo(k)fluoranthene	21.62 ± 2.19[a]	13.22 ± 1.02[b]	19.20 ± 2.57[a]	17.78 ± 2.37[a]
Indenol (1,2,3-c.d) pyrene	15.33 ± 2.13[a]	8.48 ± 1.50[b]	13.13 ± 1.65[a]	12.39 ± 1.14[a]
Dibenzo(a,h) anthracene	3.31 ± 0.25[a]	3.06 ± 0.44[b]	4.16 ± 0.65[a]	3.67 ± 1.69[a]
Benzo(g,h,i) perylene	14.04 ± 0.48[a]	9.17 ± 1.10[b]	15.28 ± 1.93[a]	7.18 ± 0.56[b]
∑PAH	**534.83 ±16.76**	**385.60 ± 14.63**	**492.29 ± 13.68**	**422.13 ± 15.55**
∑PAH4	**108.06 ± 4.39**	**75.31 ± 5.41**	**106.92 ± 8.68**	**93.27 ± 7.95**

Arithmetic mean ± SD of triplicate experiments with different superscripts are significantly different at $p < 0.05$

Proximate and Fish Quality Indices

Fish tissue is made up of various constituents such as water, protein, lipids, minerals, and fibre. These constituents are responsible for determining the nutritional value, texture, and sensory quality of fish. Table 6 presents the nutritional constituents of frozen and smoked mackerel. After smoking, the moisture content of defrosted fish samples reduced to 29% for processor 1 and 23% for processor 2. Moreover, the protein composition of frozen mackerel decreased by 13% for processor 1, but there was no significant difference observed for processor 2. There was no significant difference (P<0.05) observed in fat, fibre, carbohydrate, and ash content before and after fish smoking for both processors.

Table 6: Proximate composition of frozen and smoked mackerel after and after HACCP

	Before				After			
	Frozen [1]	**Smoked [1]**	**Frozen [2]**	**Smoked [2]**	**Frozen [1]**	**Smoked [1]**	**Frozen [2]**	**Smoked [2]**
Moisture (%)	63.44±2.43[a]	48.98±0.62[b]	63.03±1.55[a]	48.55±1.55[b]	72.38±1.91[a]	42.55±3.29[b]	67.53±5.41[a]	60.34±6.47[a]
Protein (%)	56.16±1.26[a]	48.35±1.00[b]	58.86±1.63[a]	55.05±2.68[a]	63.67±0.86[a]	62.10±0.92[a]	59.98±1.28[a]	59.52±2.39[b]
Ash (%)	2.88±0.31[a]	3.88±0.62[b]	3.42±0.13[a]	3.86±0.44[a]	3.59±0.27[a]	4.26±0.52[a]	3.51±0.26[a]	3.53±0.28[a]
Oil (%)	18.52±2.29[a]	16.88±2.48[a]	18.30±5.48[a]	16.52±5.19[a]	11.51±1.96[a]	11.21±1.58[a]	15.33±4.9 6[a]	11.70±5.19[a]
Fibre (%)	0.12±0.01[a]	0.14±0.01[a]	0.12±0.01[a]	0.15±0.02[a]	0.25±0.02 [a]	0.33±0.06[b]	0.26±0.02[a]	0.30±0.07[a]
Carbohydrate (%)	21.32±4.10[a]	31.74±3.16[b]	19.31±7.10[a]	24.41±7.42[a]	21.00±1.33[a]	22.09±0.52[a]	21.32±2.45[a]	19.55±3.78[a]

Values are means ± SD of triplicate determinations.
Mean values with different alphabet superscript before and after GMP/HACCP are significantly different at $p < 0.05$

CHAPTER FIVE

DISCUSSION

Implementation of GMP on Fish Smoking Process

Fish smoking process refers to the method of preserving fish by smoking it. It involves openly displaying fish to smoke from burning wood or other materials, which imparts a smoky flavour and helps to dry out and preserve the fish. This process has been used for centuries to extend the storage life of fish and other meats and is still popular today. In Ghana hot smoking procedure is mostly employed in mackerel processing since this smoking method can retain the tasty juice from the fish as well as colour, and texture. However, it has been observed that many local fish processors lack education in food hygiene, which results in poor food safety practices. To improve the quality and safety of smoked fish, it is necessary to develop a simplified safety protocol that can easily be integrated into traditional fish processing methods.

To achieve this, gaps in the traditional hot smoking process were identified through the observation of the entire fish processing value chain without interference, and records were taken accordingly to construct a decision tree (Figure 4) to identify the hazards as well as CCPs based on the Codex Alimentarius Logic Sequence guidelines (Table 1).

Upon arrival at the processing site, the frozen samples were rinsed once with water to remove blood, slime, and any other foreign material, which also helped to hasten the thawing process. After washing, the partially thawed fish samples were racked to drain and remove excess moisture, which decreased smoking time. The draining process can be performed either in the

open sun or in smoke generated from the kiln, at the processor's discretion. Next, the fish were hot smoked for 1-2 hours using the Chorkor kiln, after which they were allowed to cool before packaging.

The concept of fish quality involves safety, nutritional value, and availability. Fish after being caught is susceptible to spoilage because of their high moisture content, neutral pH, high amounts of amino acids, polyunsaturated fats, and the presence of naturally occurring autolytic enzymes (Stratev *et al*., 2015). Poor raw fish quality has dire consequences which transcends to loss of important protein, foodborne infection, monetary loss along the value chain (Aboagye *et al*., 2020).

Furthermore, fish processing in the country can be affected by microbial and chemical contamination due to improper handling, smoking conditions, storage, and exposure to unsanitary open markets. This research aims to improve the quality and safety of smoked fish by implementing basic good manufacturing practices with hazard analysis and critical control points.

Microbiological Quality of Fresh Fish

Several groups of microorganisms were present on the thawed fish. The microbial diversity could originate from the water environment from which they were caught, handled, stored and packaged. The total viable counts (Table 3) were comparable to TVC of chub mackerel from previous studies (Oluwayemisi *et al*., 2019; Samaha *et al*., 2015; Sikorski & Kolodziejska, 2002). Although Orngu, (2021) showed slightly higher values (4.4-4.9 Log CFU/ g) than that of this current study, TVC counts of the fresh fish were all lower than the upper limit (6 Log CFU/ g on fish wet weight) of the acceptable levels for fresh fish (ICMSF, 1980).

The washing and draining steps appeared relevant for reducing surface contamination, which may help decrease the rate of fish spoilage. The tap water obtained from a potable source could have contributed to the residual microbial loads of the fish (Ayeloja *et al*. 2020; Kirby *et al*., 2003).

Smoking caused a reduction in coliforms and *E.coli* to values that were below detection < 1 Log cfu/ g, and these finding were supported by Oluwayemisi *et al*.,(2019) who showed the detection of pathogenic bacteria isolate such as *Escherichia coli*, indicating that, the aquatic habitats of the fish is polluted with faecal matter from sewage disposal or human activities. According to Eze *et al*. (2011) & ICMSF (1980), the upper and lower limits for the total coliform count of fresh fish is 2 Log CFU/ g – 4 Log CFU/ g. Heat and smoke generated during fish smoking effectively inactivated the microbes below the detection levels. The detection of coliforms from fish at several smoking facilities, was ascribed to post-smoking unsanitary handling (Anihouvi *et al*., 2019; Obodai, *et al*., 2011), thus, necessitating the holistic examination of the processing units to control the rate of microbial proliferation on smoked fish.

Based on pigmentation, nature of conidiospores formed and mycelia, *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Rhodotorula* spp., *Mucor* spp., were isolated from the frozen fish (Figure 5c). Unsuitable storage temperature, amongst other factors such as handling, packaging, and arid environments, are prerequisites for fungal growth (Dutta *et al*., 2018). The presence of yeast and molds after smoking could be ascribed to incomplete dehydration of fish after smoking. The occurrence of *Aspergillus* spp. and *Penicillium* spp. in fish can lead to serious health problems because of their

mycotoxigenic potentials and penicillic acid (Samaha *et al*., 2015). The occurrence of molds and yeast in smoked fish has been reported (Olaolu & Olusegun A. Oyelese, 2019). Similarly, Obodai *et al*., (2011) report of yeast and mold counts from smoked fish in the range from 0 to 8.5 $\times 10^3$cfu/g. Air from the environment is the main source of yeast and molds contamination in food and contact surfaces. Traditional fish processing sites are predisposed to dust, flies, and pest invasion due to lack of proper operating facility, therefore, food and contact surfaces easily get contaminated (Asiedu *et al*., 2018)..

GMP and HACCP Implementation Outcome

Traditional fish processing in Ghana is dominated by women who mostly operate as small-medium scale. Although their role is vital, these women have restricted access to capital compared to their male counterparts (Hasselberg, 2020). Overestimation of operational cost and lack of low education are among the barriers affecting the adaptation of food safety guidelines by small to medium fish processors (Addo Opare, 2019). The concept of GMP and HACCP must be simplified to a level that can easily be adapted into the steps of traditional fish processing.

Applying the GMP and HACCP procedure led to lower TVC counts for P1 and P1. Table 3 shows that after washing, draining, and smoking, the counts were below the detection levels (<1.0 Log cfu/g). Although lower TVCs were observed in the initial microbial loads of fish, thorough washing of the drying racks using detergents and disinfectants such as 0.1% sodium hypochlorite to eliminate dust, oil and fish residues from previous smoked fish could have contributed to the lower microbial loads (Table 3). Secondly, fish was drained in an insect-free enclosed space rather than in the open air, which

could have reduced contamination from flying insects. Handling time for smoked fish during packaging was reduced to the barest minimum, to decrease the likelihood of contamination and the fish was allowed to cool while in the kiln before packaging. The wood smoke is known to contain several antimicrobial compounds including formaldehyde, acidic volatile gasses, among others that are inhibitory to the microflora (Ogbadu, 2014). Fish processors washed their hands using soap under running water after which gloves were worn before packaging the smoked product, packing into a brown clean envelop and storing under refrigeration temperature of 4℃.

Further, peroxide value (PV) of oil extracted from the smoked fish samples decreased significantly ($p < 0.05$) after applying GMP/HACCP (table 2). Even though a decrease in PV was observed, this value is however slightly higher than the recommended level 5 meq/kg (WHO, 2017). Despite the observed decrease in PV, the value is still slightly higher than the recommended level of 5 meq/kg. The high peroxide value could be attributed to increased temperature and exposure to oxygen during the process of fish smoking, which results in oil oxidation (Fuadi *et al*., 2014; Tenyang *et al*., 2020).

Histamine is the most frequently reported biogenic amine (BA) in fish and is produced when microorganism released the enzyme decarboxylase that decarboxylate free histidine (Visciano *et al*., 2014). Histamine is hardly present in freshly caught fish hence, elevated level indicates poor quality of fresh fish, contamination, and improper handling during food processing and storage (Ruiz-Capillas & Herrero, 2019). Histamine forming bacteria (*Enterobacteriaceae*. *Pseudomonadaceae*, *Klebsiella pneumoniae*, etc) can

grow rapidly at moderately abused temperatures (Visciano *et al*., 2014). Hence, the occurrence of histamine in fish or fishery products is a measure of fish putrefaction by microbial spoilage and enzyme activity (Debeer *et al*., 2021). Fish smoking temperature above 80℃ can inactivate the enzyme histidine decarboxylase and histamine forming bacteria. Nevertheless, histamine once formed is heat tolerant and so will remain in heat processed foods. Thus, the measurement of histamine levels in fish is regarded as a quality index and is an important parameter for sanitary examination (Zhai *et al*., 2012). It's been known that consumption of biogenic Amines in food can affect human health and wellbeing acutely (Debeer *et al*., 2021; Ferreira *et al*., 2020).

Notably, the US Food and Drug Administration (FDA) and European Union (EU), recommend 50mg/kg and 100mg/kg respectively as acceptable levels of histamine in edible fish (Debeer *et al*., 2021). The Codex standards, which Ghana is a member, has established acceptable histamine levels of 100mg/kg in fish and fish products (Weremfo *et al*., 2020). From this study, hot smoked mackerel had average histamine levels (Table 4) reduced significantly ($p < 0.05$) after implementing a basic GMP/HACCP procedure. The low histamine levels in the smoked mackerel investigated the low temperature preservation of the raw fish as freeze storage of fish lowers the chances of histamine production (Ahmed *et al*., 2012; Park *et al*., 2017).

Wood or plant material used for the generation of smoke is known to produce toxic substances called Polycyclic Aromatic Hydrocarbons (PAH) (Codex Alimentarius, 2009). There are over 30 unique PAHs in addition to other compounds, but Benzo[a]pyrene (BaP) is recognized as an indicator of

total PAH in smoked foods (Simko, 2009). PAHs are known to contain carcinogenic and genotoxic compounds hence, foods processed through drying, roasting, grilling and smoking techniques have varying quantity of PAHs deposits them (Asiedu *et al*., 2018; Bomfeh *et al*., 2019). Additionally, the formation of PAH on food processed by wood combustion is dependent on the choice of wood, temperature, length of smoking, distance from food to heat and fat content of the food (Adejare, 2017; Amponsah, Kjellevold, *et al*., 2020; Codex Alimentarius, 2009).

Notably, the chorkor or the metal drum is the commonly used type of oven used by traditional smokers in Ghana because they are easy to construct with little capital (FAO, 2021; Sakyi *et al*., 2019). However the use of traditional smoking oven for food processing is notorious to result in elevated levels of PAHs (Bomfeh *et al*., 2019). The European Union's maximum limits for benzo[a]pyrene is 2 µg/kg and other PAH4 (sum of benzo[a]pyrene, chrysene, benzo[a]anthracene and benzo[b]fluoranthene) is 12 µg/kg for smoked fish products (Asamoah *et al*., 2021). This proposed PAH4 concentration is considered as the most appropriate indicator of PAH occurrence in food. (Ogbadu, 2014; Stołyhwo & Sikorski, 2005). A study by Amponsah, et al., (2020) found that the use of the Chorkor smoke oven in smoking fish resulted in varying levels of PAH. This was attributed to excessive smoke and heat generated by the oven. However, the implementation of GMP/HACCP resulted in a reduction of PAH4 levels in the smoked fish. Despite this improvement, the observed levels were still higher than the recommended levels by FAO, (2019). As a result of this limitation,

the Ahotor oven was constructed as an improved alternative to the chorkor oven by Kwarteng, (2016).

Physiochemical and Proximate Composition

Physiochemical quality

Physiochemical changes of frozen mackerel after smoking is as presented in Table 5. The average weight range of thawed mackerel was 228g to 352g. After smoking, the weight significantly decreased to 109g to 297g, respectively. The average length of mackerel was in the range of 30cm to 34cm and after smoking, the length recorded was 25cm to 33cm. This is similar to FuKhedkar & Jadhao, (2003) who reports of an adult mackerel raw is about 30–35cm long, weighs 300–500g.

Food products have varying pH values depending on their constituents. The pH of a system is associated with concentration of hydrogen ions and plays a key influence on shelf life. Additionally, the pH value of fish muscle can be related to reduced glycogen level, where anaerobic glycolysis predominates (Murthy & Jeyakumari, 2004). A previous finding in atlantic salmon indicates that the muscle contraction in living muscle before post-mortem leads to lactic acid production which temporally reduces pH (Liu *et al.*, 2020).

There was no significant difference in pH between frozen and hot smoked fish. This was confirmed by researchers (Baten et al., 2020), indicating that smoking does not affect the pH value..

The brix value is a measure of sugar content in a solution and is commonly used to measure sweetness in fruits and vegetables (Barron *et al.*, 2021). Fish muscle typically does not contain sugar, hence no significant

difference was observed comparing the brix value of frozen to smoked fish.

Similarly, texture is regarded as an essential quality attribute of thermally cooked foods. Heat and smoke applied to fish decrease the water-holding capacity of fish tissue as reported by Puke & Galoburda, (2020). After smoking, no significant difference was recorded for fish smoked before and after GML/HACCP implementation since the fish had no loss in muscle tenderness. This was because mackerel was hot smoked to achieve minimum water loss, to attract a high market premium.

After a few minutes of smoking, condensation begins with colour changing from yellow to a brown colour that increasingly becomes dark brown. The colour development of smoked fish is primarily due to Maillard reaction where a longer smoking process results in a change colour (Leksono *et al*., 2020). The initial brown index for raw mackerel was in the range of 12.45 - 25.69 and after smoking 44.06 – 127.72. After smoking, the colour intensity of smoked fish was three times the initial colour. Such a significant increase after smoking could be due to smoking depositing naturally produced chemicals through thermal breakdown of wood.

Nutritional quality

In general, fish smoking has appeared to alter nutritional quality and this change depends on the procedure employed during smoking (Ejiofor *et al*., 2020). The proximate composition of frozen and hot smoked mackerel is shown in Table 5. Moisture, protein, and oil composition were significantly (P<0.05) high in frozen fish compared to smoked samples (Ferreira *et al*., 2020). Abraha *et al*., (2018) agree with this report and further explain that smoking as a processing method has an impact on fish soluble protein. Heat

denatures the physicochemical structure of protein and can lead to a decrease in the biological availability of protein. Decreased moisture levels observed in hot smoked fish in comparison to thawed frozen fish is owed to the drying effect of heat and smoke from the smoking process (Steiner-Asiedu *et al*., 1991).

It is worth mentioning that, moisture content found in smoked mackerel during this study ranged from 48% to 60%, which is similar with the findings of preceding research conducted by Goulas & Kontominas (2005). Elevated moisture content observed in this study corresponded with low fat content, which is also like what has been observed in other marine species such as those studied by Cardinal *et al*., (2001) and Kassah, (2020). This is likely because the total weight of the fish remains relatively constant, so changes in the moisture content can alter other components, including fat content. As a result, the fat content decreases in relation to the total body weight (Ferreira *et al*., 2020).

Lipid content recorded for frozen chub mackerel and smoked mackerel had no significant difference. According to Ackman & Eaton, (1971) marine fish like mackerel is classified as fatty with high fat (> 8). The lipid composition in this research is significantly high. The variations in the composition depend on several factors such as the size, species, life cycle, sex, diet, and environmental factors like season, temperature, salinity, geographical location, and whether the fish were farmed or caught in the wild (Balami et al., 2019).

Crude fibre and ash content in smoked fish offer valuable information about its nutritional value, quality, and processing. Monitoring these

components is essential for both producers and consumers to ensure the product meets nutritional requirements. No significant differences were observed in the crude fibre and ash content of fresh mackerel and smoked mackerel. This indicates that the smoking process did not affect the nutritional composition of the fish. Similar trends were observed by Nnaji & Ngele (2016) and Pop & Frunză (2015).

Carbohydrate composition in fish is primarily calculated by subtracting the percentage of water, protein, fat, and ash from 100. No significant difference was found in carbohydrate content comparing frozen to smoked fish processed by both fishmongers (p <0.05). Although mackerel is known for its high protein and low carbohydrate content, the samples analysed in this study had high levels of carbohydrates. This increase in carbohydrate content could be due to the fish's diet, as some aquaculture systems include carbohydrates in fish meal to enhance growth and meet nutritional goals such as protein and lipids (Liu *et al*., 2020).

CHAPTER SIX: SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Summary

The research investigated the current procedures used in traditional fish smoking that require hygienic improvement in Cape Coast. It also aimed to identify microbial contaminants in fish processing and use basic good manufacturing practices and Hazard Analysis Critical Control Points to improve the safety of traditionally processed fish in Cape Coast.

This research was conducted in two stages where stage one involved the evaluation of the general working condition through visual observation and the collection of samples for microbiological analysis. Stage two included the implementation of corrective steps to ensure conformity with the guideline highlighted in stage one, good manufacturing practices, and further microbiological analysis to determine the efficacy of the implemented changes. Bacterial and fungal microbes were cultured on Nutrient Agar, Violet Red Bile Glucose Agar, Eosin Methylene Blue Agar and Sabouraud Dextrose Agar. Physiochemical analysis done for the raw and smoked were weight, length, pH, texture, colour, peroxide value. In addition, proximate, histamine and PAH levels were determined.

Generally, total viable count for the thawed sample were high, followed by total fungal, coliforms, and *E.coli* count. The washing and draining step showed a reduction in microbial load for both fish processors. Although, coliforms, *E.coli*, and fungal microbes are the major microflora of smoked fish, traditional smoking results in loads that were below detection limits. After implementing the HACCP intervention procedure, the initial

TVC in thaw fish were lower. Additionally, TVCs, coliforms, *E.coli*, and fungal microbes remained low and below after fish smoking.

While histamine levels in frozen and smoked mackerel were much lower than standard hazard levels, peroxide value were higher than recommended levels. Additionally, PAH4 were not detected in the smoked mackerel. In addition, the implementation of GMP/HACCP did not significantly affect the nutritional composition, indicating fish nutritional quality is not adversely affected during traditional processing.

Conclusion

To enhance smoked fish quality and safety, this study sought to identify gaps in traditional fish processing, determine the impact of smoke on the nutritional and physicochemical quality of fish, and implement and assess the impact of basic Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP).

The major gaps identified in fish smoking is related to the smoking temperature and post-smoking contamination. The implementing basic GMP/HACCP procedures, the occurrence of microbial contaminants such as coliforms, *E. coli*, and fungi in smoked fish, was significantly reduced to acceptable levels. However, peroxide value and PAH4 levels were still quite high both before and after the GMP/HACCP procedure.

The smoking process did not affect the nutritional composition or histamine levels of the fish, it is important to note that the implementation of basic GMP/HACCP procedures has helped in improving the quality and safety of smoked fish regarding microbial contamination and physicochemical properties.

Recommendations

1. It is important to conduct further studies on the microbiological quality of smoked fish across various regions in Ghana, accompanied by the implementation of GMP/HACCP basic systems for quality control. This study was conducted only with two traditional fish processors; therefore, more informative results can be obtained by including a larger sample size.
2. Encourage fish processors to participate in food safety support groups to enhance safety and quality of smoked fish for local and international sales.
3. Financial institutions should provide flexible funding to traditional fish processors to facilitate easy adaptation to GMP/HACCP.
4. Future research should consider using freshly harvested fish for smoking to account for potential contributions of fish storage conditions to processed fish's chemical levels.

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