



COLLEGE OF BASIC AND APPLIED SCIENCES

SCHOOL OF BIOLOGICAL SCIENCES

**ASSESSING THE EFFICIENCY OF TWO IMPROVED LESSER
KNOWN KILNS AND THEIR EFFECT ON THE QUALITY AND
SHELF LIFE OF SMOKED FISH IN GHANA**

BY

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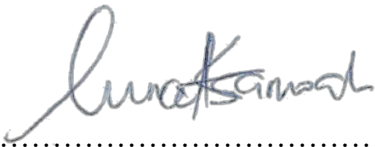
**THIS THESIS IS SUBMITTED TO UNIVERSITY OF GHANA, LEGON,
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DECLARATION

This thesis is the result of original research work undertaken by Eunice Konadu Asamoah, of the Department of Marine and Fisheries Sciences, University of Ghana, under the supervision of Prof. Francis K. E. Nunoo, Dr. Samuel Addo and Prof. Grethe Hyldig. This research has not been included in any thesis or dissertation submitted to any other institution for a degree or any other qualifications. Authors whose works were used have been duly referenced/recognised.



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ABSTRACT

Fish smoking is a traditional fish preservation method which is affordable, and hence employed in most developing countries with logistical challenges in preserving fresh fish for marketing. Smoked fish is a major source of protein in the diets of Ghanaians. Often traditional kilns, that rely on firewood as a source of fuel, are used. These kilns have been shown to be less fuel efficient and the smoked products have high levels of polycyclic aromatic hydrocarbons (PAHs), which are of public health concern. Additionally, poor storage of the products leads to quality losses. This study, which contributes to the search for a more efficient and safe smoking oven, therefore sought to test and compare the efficiency of two improved, but lesser known kilns, the Cabin and Abuesi gas fish smoker (AGFS) to the traditional Chorkor smoker by investigating the physicochemical, microbial and sensory qualities of the smoked products. Finally, the effect of irradiation and different storage conditions on the shelf life of the smoked products was studied. The research was undertaken in Abuesi, in the Western Region, using two marine fish species, the chub mackerel and barracuda.

The results showed that the AGFS had 12% lower yield but 86% and 60% higher processing rate than the cabin and Chorkor kilns respectively. Again, the fuel consumption was 68% and 54% better than the Cabin and Chorkor respectively, while the Cabin also saved 29% more fuel than the Chorkor. In terms of the fuel costs, the Cabin was 38% and 54% lower than the Chorkor and AGFS respectively (owing to the lower cost of firewood, compared to LPG). The cost of construction was however extremely high in the AGFS than the Cabin and Chorkor kilns, however, its industrial size (500 kg capacity), faster smoking time and lower fuel consumption make it good alternative to consider.

Smoking improved the physical, chemical, microbiological and sensory quality of mackerel and barracuda. These qualities, except for colour and sensory analysis, could not be statistically differentiated between the products from the AGFS and Cabin kiln. The Cabin-smoked products had the more traditional qualities of smoked fish (appearance, odour and flavour), while the gas-smoked products had a pronounced fried appearance and taste.

The AGFS produced smoked products with mean benzo(a)pyrene and PAH4 concentrations below the EU MLs (2 and 12 µg/kg respectively). Depending on the type of firewood used, the Cabin also produced benzo(a)pyrene below the MLs when *C. mildbraedii* (Esa) was used, while the Chorkor had levels 3 to 8 times higher than the MLS. The PAH4 levels in the Cabin and Chorkor products were all above the MLs (4 and 8 times higher respectively). Based on the frequency and quantities of smoked mackerel and barracuda consumed by an average Ghanaian adult (with a life expectancy of 63 years), the potential carcinogenic risks were of least concern in the gas smoked and all barracuda samples (about 1 in 100,000 adults), moderate in the Cabin smoked mackerel (3 and 6 in 100,000 adults) and high in the Chorkor smoked mackerel (7 and 17 in 100,000 adults). Heavy metal (Hg, Pb and Cd) contamination was negligible in fresh and smoked mackerel and barracuda.

The effect of irradiation and storage temperature on the quality and shelf life of smoked mackerel showed that irradiation did not affect the nutritional quality (protein, fat, moisture and ash contents) after 65 days of refrigerated storage. The fatty and amino acid compositions were also unaffected by irradiation. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) respectively constituting about 8% and 18% of the total fatty acids. The essential amino acids also contributed about 59% of the total amino acid concentrations. The non-irradiated and irradiated smoked mackerel, stored at refrigerated temperature, were of good microbial and

chemical quality by Day 65 of storage, even though lipid oxidation and hydrolysis were affected. Keeping the non-irradiated and irradiated smoked fish at room temperature were rejected by Day 5 of storage due to insect infestation and visible mouldiness (even though most microbial and chemical qualities were good).

The results, therefore, indicates that The AGFS performed better, overall, followed by the Cabin and then the Chorkor. The kilns produced fish with good nutritional qualities. Irradiation did not negatively impact on the quality of the smoked products during the 65 days of storage, but refrigerated storage is key to maintaining quality of irradiated fish.

DEDICATION

I dedicate this thesis to my dear husband, Dr. Richard Asamoah, our sons (Jayden, Joel and Jeremy), extended family and to all smoked fish processors in Ghana and other developing countries.

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LIST OF ABBREVIATIONS AND ACRONYMS

AGFS:	Abuesi Gas Fish Smoker
ANOVA:	Analysis of Variance
AOAC:	Association of Official Analytical Chemists
AOCS:	American Oil Chemists' Society
APHA:	American Public Health Association
AWEP:	Association of Women for The Preservation of The Environment
CCT:	Controlled Cooking Test
Cd:	Cadmium
CO:	Carbon Monoxide
CPAH:	Carcinogenic Polycyclic Aromatic Compounds
CSIR:	Council of Scientific and Industrial Research
DHA:	Docosahexaenoic Acid
DMA:	Dimethylamine
EnDev:	Energising Development
EPA:	Environmental Protection Agency
EPA:	Eicosapentaenoic Acid
EU:	European Union
FAME:	Fatty Acid Methyl Esters
FAO:	Food and Agriculture Organization
FDA:	Food and Drug Authority
FFA:	Free fatty acid
FRI:	Food Research Institute
FTT:	FAO-Thiaroye processing technique
GRATIS:	Ghana Regional Appropriate Technology Industrial Service

GSA:	Ghana Standards Authority
Hg:	Mercury
HMW:	High molecular weight
HPLC:	High performance liquid chromatography
IARC:	International Agency for Research on Cancer
IAEA:	International Atomic Energy Agency
ICMSF:	International Commission for Microbiological Specifications for Foods
ILCR:	Incremental lifetime cancer risk
ISO:	International Standards Organisation
LMW:	Lower molecular weight
LOD:	Limit of detection
LOQ:	Limit of quantification
LSD:	Least significant difference
MLs:	Maximum limits
MMW:	Medium molecular weight
MOFA:	Ministry of Food and Agriculture
MOFAD:	Ministry of Fisheries and Aquaculture Development
MUFA:	Monounsaturated fatty acids
NAFPTA:	National Association of Fish Processors and Traders
NO _x :	Oxides of Nitrogen
PAH:	Polycyclic Aromatic Hydrocarbons
Pb:	Lead
PCA:	Principal Component Analysis
PM:	Particulate matter
PUFA:	Polyunsaturated fatty acid

PV:	Peroxide value
QDA:	Quantitative descriptive analysis
SFA:	Saturated fatty acid
SO ₂ :	Sulphur dioxide
THQ:	Target hazard quotient
TVB:	Total volatile base
TVC:	Total viable mesophilic count
UNDP/TCDC:	United Nations Development Program/ Technical Cooperation among Developing Countries
UNU-FTP:	United Nations University-Fisheries Training Programme
USA:	United States of America
USEPA:	United States Environmental Protection Agency
WARFP:	West Africa Regional Fisheries Programme
WHO:	World Health Organization

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Fish and fisheries products serve as significant sources of food and incomes for numerous people, particularly those from developing countries (FAO, 2018). Fish is highly nutritious, containing approximately 70-84% water; 15-24% protein; 0.1-22% fat; 1-2%, minerals; 0.5% calcium; 0.25% phosphorus; and 0.1% vitamins A, D, B and C (Abraha, et al., 2018). Consumption of fish and fisheries products has been known to reduce the risks of cardiovascular diseases, type 2 diabetes, inflammatory diseases, certain cancers, dementia and psychological problems (Hosomi, Yoshida, & Fukunaga, 2015). The fishing industry further serves as an important economic activity, especially in Africa, where about 10% of the population are engaged in fisheries and aquaculture for their livelihoods (FAO, 2018).

In Ghana, fish and fish products are most important non-traditional export commodities, accounting for about 50% of all revenue from non-traditional exports (FAO, 2016-2019). The industry also contributes about 3% to the country's gross domestic product, employs about 10% of the population and generates an estimated USD 1 billion in total revenue each year (MOFAD, 2016; GIPC, 2019). Again, fish contributes significantly to the food security in the country. About 50% of the animal protein intake in the diet of Ghanaians (i.e. annual per capita consumption of about 19.8 kg; lower than the world, but higher than African average of 20.2 kg and 9.9 kg respectively) is derived from consumption of fish and fisheries products (FAO, 2018; MOFAD, 2018).

Ghana's fishery comprises the inland (freshwater and aquaculture) and marine subsectors. The marine subsector accounts for about 85% of the total fish catches, exploiting about 347 fish species, 17 cephalopod and 25 crustacean species (Nunoo, Asiedu, Kombat, & Samey, 2015). The small-scale (artisanal and subsistence), semi-industrial and industrial fishing fleets exploit the marine fisheries resources. The species mostly exploited are the small pelagics (making up a total of about 70% of total catches), like the round sardinella (*Sardinella aurita*), flat sardinella (*S. maderensis*), European anchovy (*Engraulis encrasicolus*), Atlantic horse mackerel (*Trachurus trachurus*), chub mackerel (*Scomber colias*), round scad (*Decapterus punctatus*) and other species such as the snappers, tunas and barracudas (*Sphyraena sphyraena*) (Nunoo, Asiedu, Kombat, & Samey, 2015; FAO, 2016-2019).

Fresh fish starts deteriorating soon after harvest, due to its almost neutral pH, high water activity, fast onset of rigor mortis and high amounts of nutrients that promote development of microorganisms (Belusso, Nogueira, Breda, & Mitterer-Dalton, 2016; de Alba, et al., 2019). It therefore requires a degree of processing to preserve and extend its shelf life for extended distribution and marketing. Preservation and processing techniques involves a reduction in temperature (e.g. freezing and chilling), heat treatment (e.g. smoking, boiling, frying and canning), decreasing available water (e.g. salting, drying and smoking) and altering the storage environment (packaging and refrigeration) may therefore be employed to ensure a safe and stable product (FAO, 2016a). Traditional preservation methods like salting, fermenting, drying and smoking account for 12% of all fish destined for human consumption, especially in Africa and Asia (FAO, 2018).

Smoking is an ancient and affordable preservation method that enhances the flavour, colour and texture of smoked products, while prolonging the shelf life of the product (Arason,

Nguyen, Thorarinsdottir, & Thorkelsson, 2014). In Ghana, many preservation methods are employed, with smoking being the most widely used (Ayinsa & Maalekuu, 2013). Smoking is used to preserve both marine (mainly common small pelagic species like sardines, anchovies, chub and horse mackerels and other species like the barracuda) and freshwater fish species (mostly tilapia and catfish). It has been estimated that about 70-80% of fish consumed in Ghana is smoked (Nunoo, Asiedu, Kombat, & Samey, 2015), with the products traditionally used in soups and sauces (UNDP/TCDC, 2001). Smoked chub mackerel is considered relatively cheap, whereas smoked barracuda is highly valued (Nunoo, Asiedu, Kombat, & Samey, 2015). Both are consumed locally and also exported, usually to the EU and USA, with smoked barracuda estimated to fetch up to EUR 8/kg in some European countries (Asiedu, Failer, & Beygens, 2018). These species were therefore selected based on the reasons enumerated.

Fish smoking in Ghana is categorized into two, small scale (who make up about 98%) and industrial (Asiedu, Failer, & Beygens, 2018). The small scale processors are mainly women, living in close proximity to coastal communities and rivers, who engage in both traditional smoking and trading of smoked fish products. They operate on small (individual or household levels with less than 10 kilns), medium (11-25 kiln) and large (more than 25 kilns, belonging to fish processors associations or co-operative) scale basis (Gordon, Pulis, & Owusu-Adjei, 2011; Nunoo, Asiedu, Kombat, & Samey, 2015). The industrial sector is mostly export-driven, with four companies currently active and certified by the Ghana Standards Authority to export smoked fish (Asiedu, Failer, & Beygens, 2018). Smoked fish is however mostly consumed locally but there is a well-developed marketing system extending into neighbouring countries like Togo, Nigeria, Benin, Burkina Faso and a high demand for these products in the EU and USA (Gordon, Pulis, & Owusu-Adjei, 2011; Nunoo, Asiedu, Kombat, & Samey, 2015). It has

been estimated that, between 2007 and 2016, about 20 tonnes of smoked fish valued at €79,000, on average, was exported from Ghana to the EU every year (Asiedu, Failer, & Beygens, 2018).

Fish in Ghana is mainly smoked using traditional kilns, with the most common one being the ‘Chorkor Smoker’. This kiln was developed by the Food and Agriculture Organisation of the United Nations (FAO) and the Food Research Institute of the Council for Scientific and Industrial Research (CSIR) and introduced in Ghana in 1969 (UNDP/TCDC, 2001). It replaced traditional smoking kilns (described in UNDP/TCDC, 2001), which were deemed environmentally and economically inefficient and affected the health of processors, most of whom are women. As of 2015, there were about 120,000 Chorkor and traditional smoking kilns in operation in Ghana (Okyere-Nyarko, Aziebor, & Robinson, 2015).

The introduction of the Chorkor smoker has however not solved these problems as it is only moderately fuel efficient (resulting in increased fuel use and therefore leading to deforestation of especially mangrove forests). It has not met the Energising Development (EnDev) requirement of 40% fuel saving potential and also offers moderate emission gains (Okyere-Nyarko, Aziebor, & Robinson, 2015). Furthermore, the fish is exposed to direct smoke from smoldering wood and this can result in the deposition of harmful substances like polycyclic aromatic compounds (PAH), dioxins, formaldehyde, nitrogen and sulphur oxides, as well as some heavy metals (Codex Alimentarius Commission, 2009). Some potentially toxic heavy metals detected in fresh and smoked fish are Lead, Cadmium, Mercury, Chromium, Nickel and Arsenic (Daniel, Ugwueze, & Igbegu, 2013; Bandowe, et al., 2014). Again, some PAHs are of human health concerns since they are carcinogenic, teratogenic and mutagenic to humans (Kim, Jahan, Kabir, & Brown, 2013). Also, smoked fish processors usually stay relatively long

in smoke-filled huts and thus develop eye, skin, lung and other respiratory problems (Flintwood–Brace, 2016).

These identified problems with smoking kilns necessitated the development of improved smoking technologies in Ghana, such as the Morrison, Ahotor and FAO-Thiaroye processing technique (FTT), among others (Entee, 2015a, b; IRI-CSIR, GSA, Kwarteng, 2016). A comparison of these kilns with the Chorkor smoker showed that none of the kilns met the Energising Development (EnDev) requirement of 40% fuel or energy saving efficiency and produced high levels of carbon monoxide (CO) and particulate matter (PM). With the exception of the FTT, all the smoked fish from the other kilns had PAH levels that were above the maximum limits for benzo(a)pyrene and the sum of four carcinogenic PAHs (i.e. 2.0µg/kg and 12.0µg/kg respectively) set by the EU (European Commission, 2011). This could be hazardous to both the processors and patrons of smoked fish products in Ghana (Aheto, et al., 2017).

These enumerated problems, therefore, make it imperative to search for other cost effective, fuel and energy efficient smoking kilns that offer lower emission gains. These interventions can help produce safe and good quality fish, with minimal deforestation, while at the same time impacting less on the health of fish processors. The need for improved smoking technologies was captured under the value chain development component of the West Africa Regional Fisheries Programme (WARFP), a World Bank project, which ended in 2018 for Ghana. The programme aimed at improving fish smoking technologies that reduce the levels of PAH (to conform to international standards) in smoked fish thereby making the product safe and reducing the impacts on women fish processors. It is also hoped that this will increase the marketability of smoked fish products and contribute to the country's economic growth (The World Bank, 2011).

Two technologies that are in line with the WARFP objectives will be evaluated in this study and compared with the Chorkor smoker. They are both existing technologies: the Abuesi Gas Fish Smoker (AGFS) and the Cabin smoker. The AGFS was designed by the Ghana Regional Appropriate Technology Industrial Service (GRATIS) Foundation, in consultation with smoked fish processors, to produce hygienic products that would meet both local and international safety requirements (Kleter, 2004). It uses agricultural wastes like sugarcane bagasse and coconut husks to impart the smoky flavour to the fish, while liquified petroleum gas (LPG) aids in the cooking and drying of the fish. This kiln has a closed design that safeguards processors from direct contact with fire or smoke, thereby minimising health problems, prevents excessive deforestation and produces better and more hygienic fish (Nunoo, Asiedu, Kombat, & Samey, 2015). Also, fish smoking proceeds faster, compared to traditional kilns and processors can perform other activities while fish is in the kiln (Nunoo, Tornyeviadzi, Asamoah, & Addo, 2019). In spite of these benefits, the kiln is not very popular in fish smoking ventures (Kleter, 2004; Nunoo, Asiedu, Kombat, & Samey, 2015).

The Cabin kiln was developed by the United Nations University-Fisheries Training Programme (UNU-FTP) and Matis Ltd. (an Icelandic Food and Biotech Research and Development company) in Iceland. It is intended for use in rural communities in Africa and is currently in use in Tanzania and Sierra Leone. It is estimated to use less firewood compared to traditional methods, with a much faster smoking time. It is an enclosed unit that ensures minimal exposure of processors to smoke and heat, while offering fish of good nutritional value (UNU-FTP, 2017).

Adoption of these technologies in Ghana could, therefore, be of benefit to the fishing industry (both for small scale and industrial processors). In lieu to this adoption, it is essential to evaluate

the quality of smoked fish produced using these smoking kilns and also, its market and economic potentials. Also, information obtained on the costs and performance of the kilns, will be very useful for consumers and businesses that will be interested in these technologies.

Apart from the problems with the smoking kilns, some problems relating to quality have also been identified throughout the fish value chain in Ghana. Loss of quality in fresh fish postharvest, resulting from microbial pathogenic contamination and spoilage, has been estimated to be between 11-17% (Akande & Diei-Ouadi, 2010). This loss was encountered by smoke fish processors immediately after purchase to just before smoking and they attributed the loss to poor or no icing of fish and long bargaining at the landing beaches and markets. During smoking with Chorkor and other traditional kilns, some fish also get burnt or drop into the fire. In addition, during packaging, storage and marketing of smoked fish, there is the development of rancidity in the product and insect infestation (Kleter, 2004; Akande & Diei-Ouadi, 2010). These losses in smoked fish were estimated at USD 60 million between 2006 to mid-2008 (Akande & Diei-Ouadi, 2010), a significantly high loss in monetary terms for the processors and the country as a whole.

To limit these postharvest losses, an investigation of the quality of fish after capture and through processing, storage and marketing is vital. Again, the use of proper packaging materials and subsequent storage at the right temperature can ensure that a safe, attractive and nutrient-rich product can be delivered to the consumer (Cyprian, et al., 2015). The use of low/medium dose irradiation (<1-10 kGy) can also be employed to lessen these losses and prolong the shelf life of fish (Arvanitoyannis & Tserkezou, 2014; Ehlermann, 2016). The reference dose for irradiation of fish, according to EU (2009) is 3 kGy, and this can guarantee a safe product with an increased shelf life (Arvanitoyannis & Tserkezou, 2014). Irradiation has

also been observed to reduce the levels of PAHs in some foods like wheat grains (Khalil, Albachir, & Odeh, 2016).

Based on the background provided above, this project was therefore designed to answer the following **research questions**:

- How will the improved kilns perform in terms of efficiency and cost effectiveness compared to the traditional Chorkor kiln?
- How will the type of smoking kiln used affect the physicochemical, microbial and sensory quality of smoked chub mackerel and European barracuda?
- How do the levels of PAHs and heavy metals in the smoked products compared with the EU standards?
- How will irradiation and different storage temperatures impact the shelf life of smoked chub mackerel?

1.2 Aim and Objectives

The main aim of this study was to assess the efficiency of the Cabin and AGFS kilns and determine their effects on the quality and shelf life of the Atlantic chub mackerel and European barracuda.

The specific objectives were to:

1. compare the efficiency of the improved smoking kilns to the traditional Chorkor smoker;
2. investigate the effect of the smoking kilns on the physicochemical, microbial and sensory quality of smoked chub mackerel and European barracuda;

3. assess the levels of PAH and heavy metals in fresh and smoked chub mackerel and European barracuda to evaluate the safety of the products for consumption and
4. determine the influence of irradiation and storage conditions on the quality and shelf life of mackerel smoked with the AGFS.

1.3 Organisation of study

This thesis is divided into seven main chapters. Chapter One gives a general introduction to the thesis. Chapter Two details a review of relevant literature. Chapters Three to Six, which tackle the stated objectives, are presented in Figure 1.1. Chapter Seven presents the conclusions and recommendations.

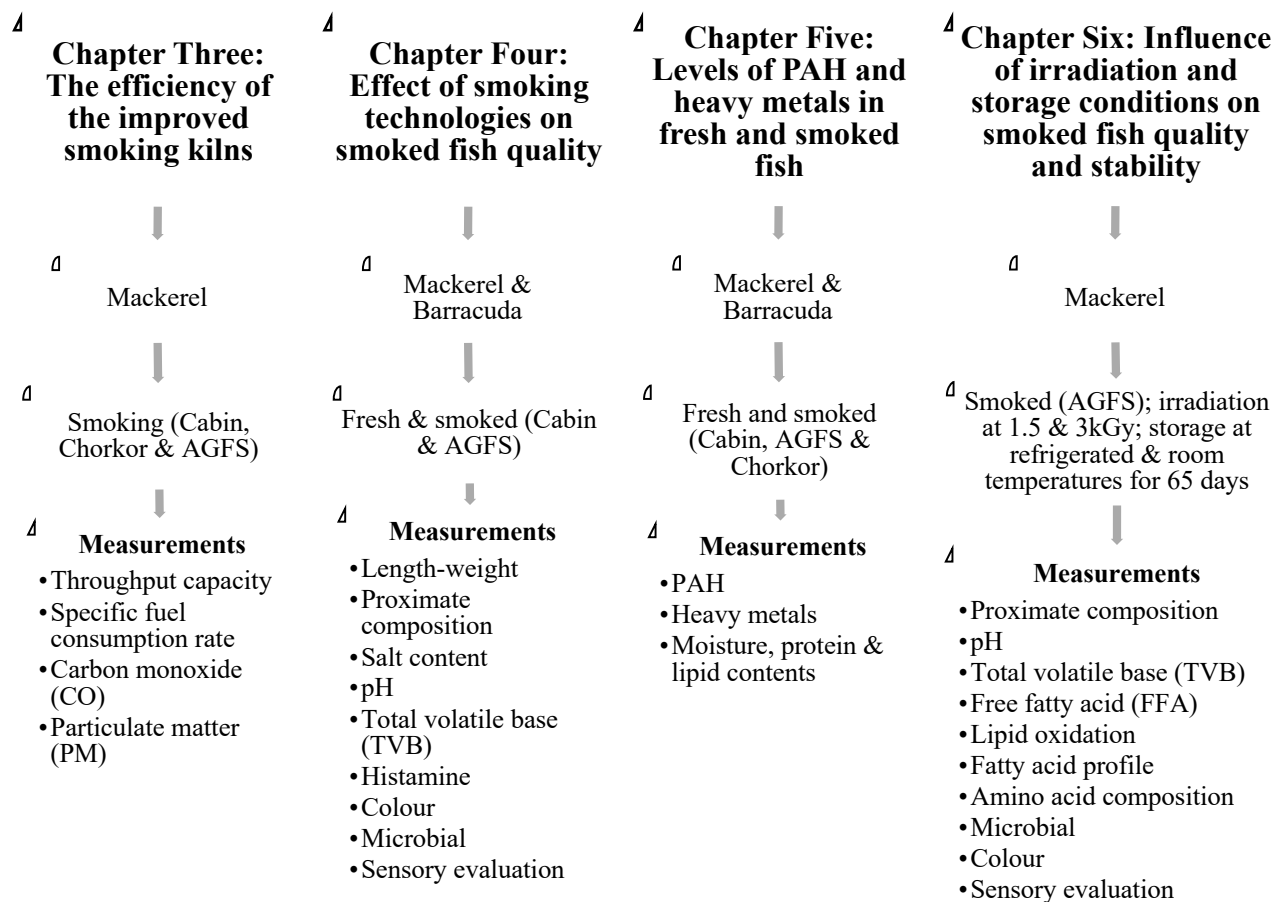


Figure 1.1: An overview of the study design for this thesis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fisheries sector in Ghana

Ghana's fishing industry is categorised into the inland (freshwater and aquaculture) and marine sectors. The marine sector contributes the most to local fish production, accounting for about 85% of the total catches, and exploiting about 347 fish species, 17 cephalopod and 25 crustacean species (Nunoo, Asiedu, Kombat, & Samey, 2015). The resources are exploited by the small-scale (artisanal and subsistence), semi-industrial and industrial fishing fleets (FAO, 2016-2019). The artisanal fleets consist of wooden canoes (about 12,847 canoes) that are either motorised or not; the inshore boats are mainly wooden with an inboard engine, while the industrial vessels are generally over 25 m long, steel-hulled and can operate in jurisdictions outside Ghana (MOFAD, 2016). Overall, the catch from all fleets averages 300,000 metric tonnes annually (FAO, 2016-2019), but this has been decreasing since 2001, while the fishing effort has been increasing (Nunoo, Asiedu, Amador, Belhabib, & Pauly, 2014).

The artisanal sector is the most significant, employing about 250,000 fishermen (about 92% of the total fishers) and accounts for 70% of total landings (FAO, 2016-2019). The sector also employs about 60% of the women involved in the fishery value chain and operates from 334 landing sites in 195 fishing towns across four coastal regions (Nunoo, Asiedu, Amador, Belhabib & Pauly, 2014; MOFAD, 2016). The artisanal sector employs multiple gears like beach seine, set net, hook and line, drift gill net, and purse seine ('ali', 'poli' and 'watsa') nets. The small pelagic species i.e. sardines, anchovies and mackerels (approximately 85% of canoe catch) are exploited with the large pelagic fish, mostly tuna and demersal stocks i.e. croakers, red snapper, sea breams and red mullet (Nunoo, Asiedu, Kombat, & Samey, 2015).

2.2 Species of interest

The species of interest are the Atlantic chub mackerel (*Scomber colias*, Gmelin, 1789) and the European barracuda (*Sphyraena sphyraena*, Linnaeus, 1758). These are important pelagic fish species that are mainly smoked and consumed locally by Ghanaians (Heinbuch, 1994). They are sold mainly unpackaged in open markets and are used in most soups and sauces (Abboah-Offei, 2016).

2.2.1 Atlantic chub mackerel

The Atlantic chub mackerel (*S. colias*) (Plate 2.1) belongs to the Family Scombridae. It is coastal pelagic and occur over the continental slope (Collette, et al., 2011). The Atlantic chub mackerel occurs in subtropical regions of the Atlantic Ocean, Mediterranean and Black Seas. It occurs at depths of 0-300 m (Riede, 2004), can grow to a length of 18 cm and may live for up to 13 years (Collette, et al., 2011). The species feeds on small pelagic fishes like anchovies and sardines, as well as pelagic invertebrates.



Plate 2.1: The Atlantic chub mackerel (*S. colias*)

The Atlantic mackerel is very important in the commercial fisheries sector throughout its range (Collette, et al., 2011). In Ghana, it is mainly exploited by the artisanal fleets, and catches fluctuate annually (Figure 2.1). Fresh mackerel has dark red flesh colour, moist and flaky

texture and rich pronounced flavour. It is a fatty fish, rich in omega-3 and unsaturated fatty acids and is also an excellent source of riboflavin, vitamins B6 and B12, protein, selenium and niacin B12 (Hyldig, Larsen, & Green-Petersen, 2007; Stadlmayr, et al., 2010; Nogueira, Cordeiro, & Aveiro, 2013; NOAA Fishwatch, 2017). It is a relatively cheap fish that is consumed by majority of Ghanaians, mainly smoked but sometimes fresh.

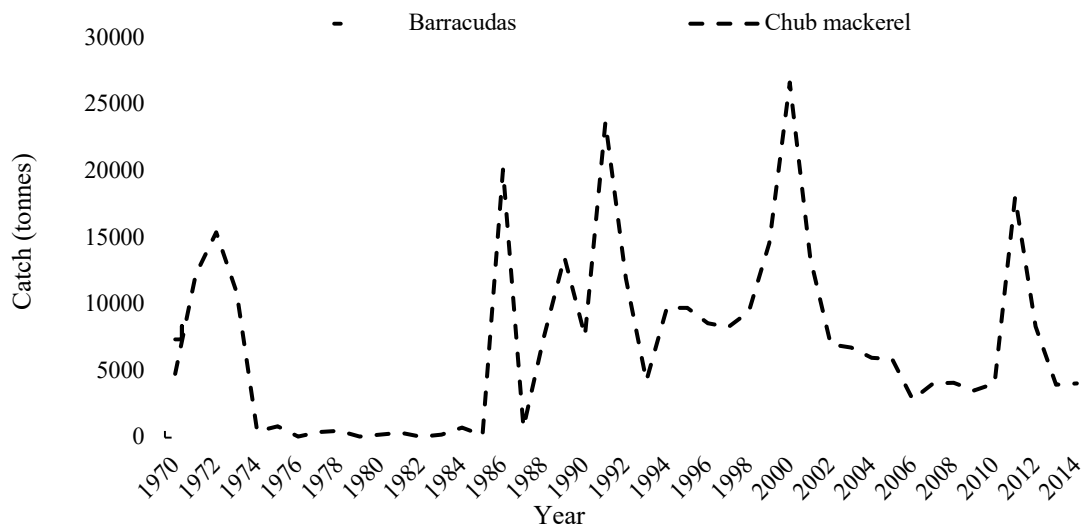


Figure 2.1: Catch trends of Atlantic chub mackerel and European barracuda in Ghana (Source: FAO FishStat)

2.2.2 European Barracuda

The European barracuda (*S. sphyraena*) (Plate 2. 2) belongs to the Family Sphyraenidae. It occurs in the epipelagic zones of coastal and offshore waters of the Eastern Atlantic (de Morais, et al., 2015). The species may be found at depths of 100 m and it is a specialized piscivore, feeding normally on pelagic and supra-benthic fishes (make up more than 99% of diet), and less frequently on cephalopods and crustaceans (Kalogirou, Mittermayer, Pihl, & Wennhage, 2012). The barracuda can grow a maximum length of 150 m and can attain a maximum age of 8 years (de Morais, et al., 2015).

The European barracuda is a commercially important fish species and it is mainly caught by artisanal and recreational fishers (Nunoo, Asiedu, Kombat, & Samey, 2015). The annual catches are generally increasing (Figure 2.1). The barracuda is a semi-fatty fish and it is rich in protein, vitamins, minerals and omega-3 and polyunsaturated fatty acids (Hyldig, Larsen, & Green-Petersen, 2007; FEP, 2007-2013; Özogul, Özogul, Çiçek, Polat, & Kuley, 2009; Stadlmayr, et al., 2010). The fresh barracuda has a light colour and tender, tasty meat (Grygus, 2015). In Ghana, it is a highly valued fish and is consumed mostly in the smoked form (Nunoo, Asiedu, Kombat, & Samey, 2015). It is also exported, usually to the EU and USA, where it can fetch up to EUR 8/kg in some European countries (Asiedu, Failer, & Beygens, 2018).



Plate 2.2: The European barracuda (*S. sphyraena*)

2.3 Fish Preservation Methods

Fish is extremely perishable and therefore requires a degree of processing to preserve and prolong its shelf life and thus allow for extended distribution and marketing opportunities (FAO, 2016a). The preservation techniques may: reduce the temperature (e.g. freezing and chilling); use heat treatment (e.g. smoking, boiling, frying and canning); decrease the water

available for microbial attack (e.g. salting, drying and smoking) and alter the storage environment (packaging and refrigeration) (FAO, 2016a). Globally, cured fish (dried, smoked, salted and fermented) makes up about 12% of fish destined for human consumption, and this contribution is mainly from Africa and Asia (FAO, 2018).

In Ghana, fish is preserved primarily by traditional (such as salting, drying, smoking and fermenting or a combination of these) and modern (freezing and canning) methods (Asiedu, Failer, & Beygens, 2018). Traditionally processed fish is widely patronized by many Ghanaians, as they are very affordable and possess good taste (Asiedu, Failer, & Beygens, 2018). Smoked fish is however the most preferred, accounting for an estimated 70-80% of all domestic catch consumed (Nunoo, Asiedu, Kombat, & Samey, 2015; Asamoah, 2018).

2.3.1 Fish smoking

Fish smoking is a process of preserving fish by subjecting it to smoke from biomass combustion, and typically includes an integration of salting, drying, heating and smoking steps in a smoking chamber (Codex Alimentarius Commission, 2013). The smoke generated adds flavour, taste, colour (sensory qualities that are desired by consumers) and preservative agents to the fish (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). Smoking can increase the shelf life of a product due to the synergistic effects of salting, drying and smoke generation (Asamoah, 2018). Salting lowers the water activity of the products, thus, inhibiting microbial growth; drying (using high temperatures) ensures a physical surface barrier is created, that inhibits the passage of microorganisms into the fish; and the smoke generated deposits compounds like aldehydes, carboxylic acid and phenols, which slows down growth of microorganisms and rancidity development (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014; Cieřlik, Migdał, Topolska, Mickowska, & Cieřlik, 2018). Raw materials of good quality

are necessary in producing high quality smoked products, that can ensure a steady market demand and profit for processors (Asamoah, 2018).

Fish products can be cold or hot smoked (or a combination of both), depending on the smoke delivery mechanism (Codex Alimentarius Commission, 2013). These methods are used both in developed and developing countries around the world, even though they are carried out mostly under controlled conditions in the developed than the developing countries (Cyprian, et al., 2015). Cold smoking occurs at temperatures generally below 30°C for about 20 hours (Doe, 1998), which leaves the fish proteins mostly intact, while at the same time, reducing the water activity (Codex Alimentarius Commission, 2013). The smoke imparts the characteristic aroma and flavour, but the final product is not cooked and thereby requires further cooking before consumption, with the exception of fish such as salmon, trout and arctic char (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014).

Hot smoking, according to Codex Alimentarius Commission (2013) exposes fish to an appropriate combination of temperature (usually 70-80 °C) and time that can cause complete protein coagulation in muscle. Hot smoking can kill parasites, impair non-sporulated bacterial pathogens and damage spores that pose health threats to humans (Codex Alimentarius Commission, 2013). The final products are completely cooked and are considered ready-to-eat (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). A drying step could be added to further reduce the water activity to about 0.75 or less so products can be stored, transported and marketed without refrigeration (Akande, Ayinla, Adeyemi, Olusola, & Salaudeen, 2012). This method is preferred in most developing countries like Ghana where refrigeration and other logistics might be a problem (Akande, Ayinla, Adeyemi, Olusola, & Salaudeen, 2012).

2.3.1.1 Changes in fish muscle during smoking

Smoking can affect the weight, pH, protein structure, flavour and texture of fish muscle (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). Weight loss results from dehydration and the leaching of lipids in fish muscle (Løje, 2007; Asamoah, 2018). This loss relates directly to the type of raw material used (whether fatty or lean fish), the final product characteristics, the dimensions of the fish and the processing parameters like times and temperature during smoking (Sigurgisladottir, Sigurdardottir, Torrissen, Vallet, & Hafsteinsson, 2000). This loss has been estimated between 10-25% and this directly affects the yield of the final product, important economic consideration that can affect the profitability of the business (Løje, 2007). Fatty fish have decreased dehydration and as such, a higher yield than lean fish (Cardinal, et al., 2004).

During smoking, the pH of the fish muscle may decrease owing to an uptake of acids from the smoke, dehydration, smoking duration and protein constituents reacting with the phenols, polyphenols and carbonyl compounds within smoke (Hassan, 1988; Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014; Lira, Silva, Figueirêdo, & Bragagnolo, 2014). The decrease in pH could result in muscle proteins having reduced net surface charge, thereby, causing proteins to partially denature, decreasing water-holding capacity and influencing the microbiota the muscle (Teixeira, et al., 2014; Bowker & Zhuang, 2015). This can further have negative effect on the texture of fish muscle, with muscle toughness increasing with decreasing pH (Huss, 1995). Espe, Nortvedt, Lie, & Hafsteinsson (2002) also reported a reciprocal relation between the smoking temperature and pH in fish muscle.

Changes in protein structure can result during the salting step of the smoking process. A low salt concentration (< 1 M) can cause a swelling of the filament lattice and depolymerization of

myosin (Cyprian, Oduor-Odote, & Arason, 2019), whereas a higher salt concentration (4 M) causes protein aggregation in the muscles (Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2011). These two phenomena can lead to variations in yield, water-holding capacity, texture and microstructure of the fish muscle (Arason, Nguyen, Thorarinsdottir & Thorkelsson, 2014). Cyprian, Oduor-Odote, & Arason (2019) further report that these conformational changes can affect the nutritional quality (i.e. loss of thermolabile compounds like amino acids) in the final product. The extent of these changes is however dependent on the food composition and the type of smoking method used; with textural changes in hot smoked fish mainly due to protein denaturation, whereas that in liquid smoking is as a result of protease enzyme activities (Arason, Nguyen, Thorarinsdottir & Thorkelsson, 2014).

The characteristic flavour, colour and odour of smoked fish results from the Maillard (browning) reaction between the carbonyl amino group, caramelization of fish flesh and lipid oxidation during the smoking process (Jaeger, Janositz, & Knorr, 2010; Leksono, Suprayitno, & Hardoko, 2014). The type of fuel used can affect these attributes, especially the colour. In Ghana for example, mangrove is mostly for smoking because it produces the characteristic golden or dark brown colour, that consumers desire (Obodai, Muhammad, Obodai, & Opoku, 2009). Smoked flavour and odour is further developed from absorption of phenolic compounds (guaiacol and syringol) produced from pyrolysis of lignin (Jónsdóttir, Ólafsdóttir, Chanie, & Haugen, 2008). An increase in temperature and dryness during fish smoking can enhance these reactions (Doe, 1998).

2.3.2 Fish smoking in Ghana

In Africa countries like Ghana, Nigeria, Cote d'Ivoire, Togo, Benin, Sierra Leone, Liberia, Kenya, Uganda, Tanzania, fish smoking is predominantly practiced by women within and

around coastal areas and along the fringes of inland water bodies (Adeyeye & Oyewole, 2016). Both fresh and frozen (local and imported) fish can be used in smoking, with the frozen fish used mostly in the lean fishing season (Entee, 2015c). The smoking process is usually in the following steps:

- preparation of fish: thawing in the case of frozen fish; then washing and sorting of the fish. Seawater is mostly used in the washing step;
- loading of fish onto smoking racks and allowing it to dry for at least 15 minutes (depending on the fish type and size) in open air environment;
- cooking and smoking of fish in/on the smoking kiln. The cooking step requires heat, and once cooked, smoke is added, usually by covering the racks and reducing the heat. The duration of these steps relies largely on the type/size/quantity of species, the smoking kiln used, the experience of the processor and the consumer preference (Asamoah, 2018).

Fish can be soft or hard smoked, based on the type and size of fish, product form, the consumer preference and the desired shelf life (Pemberton-Pigott, Robinson, Kwarteng, & Boateng, 2016). Soft smoking usually takes between 1-3 hours and yield products with moisture contents of about 40-50% (Kwarteng, Nsiah, Samey, Boateng, & Aziebor, 2016). The mackerel and barracuda are typically soft - to medium-smoked (Abboah-Offei, 2016). Hard smoking is preceded by soft smoking and the smoked products are dried afterwards for between 10-18 hours, sometimes days, depending on the weather (Kwarteng, Nsiah, Samey, Boateng, & Aziebor, 2016). The fish produced have moisture contents of between 10-15% and sometimes, lower than 10% (Pemberton-Pigott, Robinson, Kwarteng, & Boateng, 2016). The small pelagics such as sardines and anchovies are usually smoked using this method and remain readily available in many markets in Ghana (Alhassan, Boateng, & Ndaigo, 2012).

2.3.2.1 Fish smoking kilns

Fish are usually smoked using traditional kilns such as the metal drum and cylindrical or rectangular kilns fashioned from mud. These kilns however had a low throughput capacity and were fuel inefficient (Avega & Tibu, 2017). Again, the mud kilns easily wore out and the metal drums could become extremely hot, and processors suffered burns and complications from smoke exposure (Asamoah, 2018; Bomfeh, et al., 2019). These challenges led to the development of the Chorkor kiln in 1969 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, Ghana) (UNDP/TCDC, 2001). This kiln can be constructed using either cement blocks or mud and has a better product throughput and fuel efficiency, lasted longer, with less labour input required (Bomfeh, 2016). These traditional kilns, especially the Chorkor, are also used in most parts of Africa where fishsmoking is predominant (Adeyeye & Oyewole, 2016).

Other improved kilns have been developed over the years. Some of these are the Morrison, Ahotor and FAO Thiaroye Technology (FTT) (Entee, 2015c). Okyere-Nyarko, Aziebor, & Robinson (2015) reported an estimated 120,000 traditional and Chorkor kilns in operation Ghana. These kilns rely on biomass fuels (mainly firewood, charcoal and agricultural by-products like sugarcane bagasse and coconut husks) as a source combustion. Entee (2015b) reported that a substantial amount of biomass fuel is used by these kilns and the temperature, humidity and smoke generation cannot be controlled (except in the case of the FTT that uses external smoke generators). Processors using mostly the Chorkor and other traditional kilns are thus exposed to smoke, and smoked products are generally of poor quality (Kleter, 2004; Antwi-Boasiako, 2017). There is therefore the need to search for or develop efficient and safe fish smoking kilns in Ghana.

2.3.3 Chemical composition of smoke and smoked foods

Smoke is made up of about 400 identified compounds, which include polycyclic aromatic hydrocarbons (PAHs), carbonyls, acids, alcohols, esters, aldehydes, phenols, furans, lactones, dioxins, and heavy metals (Mejlholm, Andersen, & Dalgaard, 2007; Codex Alimentarius Commission, 2009). The phenolic compounds, carbonyls, aldehydes, among others give the characteristic flavour of smoked products, as well as possessing antimicrobial and antioxidant properties, as discussed above (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). The PAHs and heavy metals are however of human health importance, as such, their levels in smoked fish require special attention (Codex Alimentarius Commission, 2009).

2.3.3.1 *Polycyclic aromatic hydrocarbons (PAHs) contamination in fresh and smoked fish*

PAHs are pervasive carbon-based ecological contaminants, that are semi-volatile and made up of fused benzene rings in linear, cluster or angular arrangements (Abdel-Shafy & Mansour, 2016). They can dissolve in fish lipids and can be obtained from natural or anthropogenic sources (Stogiannidis & Laane, 2015). The natural sources are petrogenic (i.e. petroleum and coal) and biogenic (i.e. transformation of natural organic matter), whereas the anthropogenic, the major environmental contributors are pyrogenic (i.e. incomplete biomass or fossil fuel combustion) (Bandowe, et al., 2014).

There are about 660 parent PAH (Stogiannidis & Laane, 2015), and they have been classified based on the number of ring as:

- low molecular weight (LMW): two to three rings, with molecular weights (MWs) from 152 to 178 g/mol, e.g. acenaphthene, fluorene, acenaphthylene, anthracene and phenanthrene;

- medium molecular weights (MMW): four rings, with MW of 202 g/mol, e.g. fluoranthene and pyrene; and
- high molecular weight (HMW): five to seven rings with MWs from 208 to 278 g/mol, e.g. benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, chrysene (ATSDR, 1995).

Different organisations and regulatory bodies have compiled lists of priority PAHs (Stogiannidis & Laane, 2015), with the United States' Environmental Protection Agency (USEPA) list of 16 PAHs widely used. These PAHs have been also categorised by the International Agency for Research on Cancer as follows:

- Group 1 i.e. definite carcinogenic: benzo(a)pyrene;
- Group 2A i.e. probably carcinogenic: dibenzo (a,- h)anthracene;
- Group 2B i.e. possibly carcinogenic: benzo(k)fluoranthene, benzo(b)fluoranthene, chrysene, naphthalene indeno(1,2,3-cd)pyrene, and benzo(a)anthracene;
- Group 3 i.e. not classified as to its carcinogenicity to humans: acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, fluoranthene and benzo(g,h,i)perylene (IARC, 2018).

Groups 1 to 2B are mostly HMW PAHs, whereas Group 3 are the LMW PAHs.

Humans can inhale PAHs, ingest them or get if from direct diffusion into the skin (Li, et al., 2016). PAHs are carcinogenic, mutagenic and teratogenic and pose significant health risks to humans (Codex Alimentarius Commission, 2009; Xia, et al., 2010; Kim, Jahan, Kabir, & Brown, 2013; Essumang, Dodoo, & Adjei, 2012, 2014; Bandowe, et al., 2014; Ncube, et al., 2017). With prolonged exposures to sufficiently high concentrations, these risks intensify especially in adults than in children (with a ratio of 3:1), as reported by Alomirah, et al. (2011).

PAHs can occur in the marine environment and as such in fish. Bandowe, et al. (2014) and Nyarko, Botwe, & Klubi (2011) found PAHs in fresh *Cynoglossus senegalensis*, *Pomadasys peroteti*, *Drapane africana*, *Sardinella maderensis* and *Galeoides decadactylus* from Ghana's coastal waters. According to European Commission (2011), however, these PAHs do not accumulate in fresh fish muscle, as they are rapidly oxidised and metabolised. No limits have therefore been set for fresh fish, however, the levels in fish can signify environmental contamination (Bouloubassi, et al., 2006).

During smoking, PAHs adsorb to or condense on the surface of the products, with the amounts dependent on the:

- type of fuel (i.e. biomass, fossil fuel, liquid or solid waste and others);
- direct or indirect smoking or drying method;
- process of smoke generation, either from smoke generator, or from using smoke condensates;
- the distance and position of the fish relative to the heat source;
- fish lipid content and how it changes during smoking;
- time and temperature of smoking and direct drying;
- hygiene and maintenance of equipment;
- the density of the smoke in the smoking chamber (Codex Alimentarius Commission, 2009)

The importance of PAHs in muscle meat of smoked fish products has been recognised by the European Commission, which has prescribed maximum limits (MLs). These MLs pertain to benzo(a)pyrene and the sum of four carcinogenic PAHs (benzo(a)pyrene, chrysene, benzo(a)anthracene and benzo(b)fluoranthene) have been set at 2.0 and 12.0 µg/kg respectively

in muscle meat of smoked fish (European Commission, 2011). There have been various studies relating to PAHs in smoked fish in Ghana, with most of the levels higher than that of the EU MLs (Table 2.1). The results from studies indicated that the type of smoking kiln, smoking method (hard/soft smoked), fuel and fish species could affect the levels of PAHs in smoked fish.

The health risks resulting from consumption of fish contaminated with PAH can be assessed using the benzo[a]pyrene equivalent (B(A)P_{eq}), incremental cancer risk and target hazard quotients (THQ) (Bandowe, et al., 2014; Xia, et al., 2010; Li, et al., 2016). The B(A)P_{eq} estimates the overall toxicity of the PAH compounds in food based on their individual concentrations and potency equivalency factors (PEFs). The cancer risk (CR) is then calculated using the B(A)P_{eq}, average fish ingestion rates, life expectancy and body weight of the target population, and carcinogenic potency of benzo[a]pyrene (Bandowe, et al., 2014; Xia, et al., 2010; Li, et al., 2016). THQ is also calculated as the ratio of estimated dose to oral reference dose (RfDo) and measures the non-carcinogenic risk from PAH exposure. A CR of 1×10^{-5} is considered the carcinogenesis threshold, with those $\geq 1 \times 10^{-4}$ deemed serious and therefore requiring attention whereas; $THQ < 1$ signifies negligible health risk (USEPA, 2004; Xia, et al., 2010; Essumang, Dodoo, & Adjei, 2013; Bandowe, et al., 2014).

Table 2.1: PAH concentrations in smoked fish from different kilns in Ghana

Fish type	Kiln type	Levels ($\mu\text{g}/\text{kg}$)		Reference
		Benzo(a)pyrene	PAH4	
Mackerel	Chorkor	41.3	452.3	Esumang, Dodoo, & Adjei, (2013)
		1.3**	168.4	
		15.2***	225.8	
Cigar minnows		0.5	50.7	
		3.0**	57.5**	
		2.9***	6.8***	
Tuna		5.9	22.9	
		0.6**	24.7**	
		0.6***	35.3***	
Sardinella		16.6	40.7	
		1.4**	17.5**	
		1.6***	43.0***	
Sardinella	Chorkor	22	84	IRI-CSIR, GSA, & Kwarteng (2016)
	Morrison	30	110	
	Ahotor	5.9	53.1	
Barracuda (soft-hard smoked)	*FAO FTT	0.6-1.8	3.6-7.6	Bomfeh, et al., 2019
	Chorkor	50.3-61.1	270.3-	
	Metal drum	37.4- 69.8	360.3 167.5- 327.1	
Sardinella (soft-hard smoked)	*FAO FTT	0.2-0.3	1.5-2.2	
	FAO FTT	1.9 & 7.7	37.0 & 28.9	
	Chorkor	26.4-60.3	166.9-	
	*Chorkor	10.2	394.5	
	Metal drum	11.1- 25.6	39.4 58.1-135.7	
Tuna	Chorkor	26.7	195.5	Nunoo, Tornyeviadzi, Asamoah, & Addo (2019)
Barracuda		71.1	865.7	
Grouper		50.3	365.8	
Tuna	Abuesi gas fish smoker (AGFS)	1.4	8.2	
Barracuda		ND	4.7	
Grouper		ND	0.3	

* = charcoal; ** = sugarcane bagasse; *** = mangrove; ND = not detected

2.3.3.2 Heavy metal contamination in fresh and smoked fish

Heavy metals are naturally occurring elements of the earth's crust or can be introduced into the environment via anthropogenic means, such as biomass and fossil fuel combustion, urban runoffs, agricultural activities, among others (Bandowe, et al., 2014). Some potentially toxic heavy metals detected in fresh and smoked fish are Lead (Pb), Cadmium (Cd), Mercury (Hg), Chromium (Cr), Nickel (Ni) and Arsenic (As) (Doe, 1998; Daniel, Ugwueze, & Igbegu, 2013; Bandowe, et al., 2014). IARC (2018) has classified Cd, Pb and Hg as Group 1, 2B and 3 carcinogens, respectively.

Heavy metals cannot be metabolised by the human body and as such bio-accumulate in the liver, muscles and bones (Ekpo, Asia, Amayo, & Jegede, 2007). Bioaccumulation of these metals in humans can result in difficulties in swallowing, muscle cramps, hypoxia, diarrhea, and in the worst event, death (Abboah-Offei, 2016). The EU has thus set the limits of Cd in muscle meat of mackerel at 0.10 mg/kg and other fish at 0.05 mg/kg; that for Pb and Hg are 0.30 and 0.50 mg/kg respectively (European Commission, 2015). Again, the carcinogenic risks are estimated using target hazard quotients (THQ) as described above (Bandowe, et al., 2014). According to Abboah-Offei (2016), As and Pb were not detected market samples of smoked tilapia, mudfish, tuna and mackerel, whereas Hg and Ni were detected at levels above the EU MLs. Bomfeh (2016) also reported levels above the EU MLs for Hg and Cd in market samples of smoked sardines. These results from market samples could however be as a result of contributions from other sources, in addition to those from fish smoking, as suggested by Abboah-Offei (2016). Bandowe, et al. (2014) however reported THQs < 1 (negligible risks associated with consumption) for three fresh fish species found in Ghanaian waters.

2.3.4 Quality degradation of smoked fish

During the storage life of smoked products, the problems of microbial growth and lipid oxidation arise. This can happen irrespective of the storage conditions and packaging used, although the rates may differ (Cyprian, et al., 2015). Akande & Diei-Ouadi (2010) have estimated quality losses in the smoked fish industry in Ghana as 37.5% of the total production.

2.3.4.1 Microbial spoilage

Healthy, live, fish have considerably sterile muscles, but the surfaces of the skin, gills and gastrointestinal tracts could harbour microorganisms (Gram, 2009). Upon death, the fish's body resistance is impaired, and this causes opportunistic microorganisms to flourish, with the enzymes they secrete further degrading the quality of the fish (Bataringaya, 2007). Processing methods like smoking, drying and salting can inhibit but not completely eliminate the effects of some of these microbes (Asamoah, 2018).

Smoked products are stored at ambient temperatures and sold unpackaged in open air markets (in most developing countries like Ghana), making them susceptible to microbial infestation, as well contamination from dust and insects (Kleter, 2004). The shelf life of soft and hard smoked fish has been estimated at 1-3 days and 6-9 months (with periodic re-smoking) respectively (Pemberton-Pigott, Robinson, Kwarteng, & Boateng, 2016).

The actions of microorganisms can result in undesirable effects on food (Figure 2.2). Bacteria such as *Aeromonas*, *Alteromonas*, most *Enterobacteriaceae*, *Shewanella*, *Vibrio* and *Photobacterium* are capable of decomposing proteins and other nitrogenous compounds (trimethyl amine oxide, TMAO) present in fish (Ryder, Karunasagar, & Ababouch, 2014). This breakdown produces trimethylamine (TMA), dimethylamine (DMA) and ammonia, which give

off the typical off-flavours and odours of spoiled fish (Bataringaya, 2007). The levels of TMA, DMA and ammonia can be measured using the total volatile base (TVB) as a quality index (Bataringaya, 2007). The temperature, time, species, hygienic condition, microbial action, among other factors, can influence the development of TVB in fish (Oehlenschläger, 2014).

The nutritional quality of fish can be damaged by biogenic amine (BA)-producing bacteria such as *Staphylococcus* spp., *Morganella morganii*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Clostridium* spp., *Vibrio alginolyticus*, *Pseudomonas putida*, *Aeromonas* spp., among others (FAO/WHO, 2013). BAs are low MW organic bases, formed in fish (usually upon death) by either the removal of the carboxyl group of corresponding amino acids by decarboxylase enzyme or the by the transamination of aldehydes and ketones (Zhai, et al., 2012; Huang, Wang, Wang, Hu, & Chui, 2019). The BAs of significance are histamine, cadaverine, tryptamine, tyramine and putrescine, which are derived from the amino acids, histidine, lysine, tryptophan, tyrosine and ornithine, respectively.

Histamine is produced by bacteria usually present in the marine environment; and inhabit the gills, skin, and gut of live fish, without causing harm to the fish (Visciano, Schirone, Tofalo, & Suzzi, 2012). However, once the fish dies the bacteria can grow rapidly, particularly following time/temperature abuse, especially in most developing countries where the cold chain after fish harvest is often poor (FAO/WHO, 2013; Asamoah, 2018). Processing methods like freezing, canning, smoking, among others, can deactivate the bacteria responsible for histamine formation, but the enzyme, histidine decarboxylase, already present in fish cannot be eliminated by these methods (FDA, 2011). Quantifying histamine levels in fish therefore provides a quality index that that can be used for sanitary surveillance (Zhai, et al., 2012; Gopalapura, Gowda, Narayan, & Gopal, 2016). Species belonging to the family *Scombridae* (mostly tuna and mackerel) are most susceptible, with other non-scombroid fish species such

as those from the *Clupeidae*, *Engraulidae*, *Coryfenidae*, *Pomatomidae*, *Scombrosidae* families also at risk (FAO/WHO, 2013). Histamine poisoning can occur from within minutes to an hour, following consumption and can last from 12 hours some days (FDA, 2011). The main symptoms may include peppery or metallic taste, numbness of the mouth, headache, vertigo, palpitations, drop in blood pressure, swallowing difficulties and dehydration (Visciano, Schirone, Tofalo, & Suzzi, 2012).

Certain pathogenic bacteria have been implicated in seafood-related illness in humans. These microbes can cause food-borne infections (from directly consuming live pathogens in food) and food-borne intoxications (resulting from ingestion of toxins produced by bacteria in food), depending on levels of pathogens and food matrix (Morris & Potter, 2013). Examples of pathogens causing infections are *Listeria monocytogenes*, *Salmonella* sp., *Escherichia coli*, *Vibrio vulnificus* and *Shigella* sp. while those that cause intoxication include *Staphylococcus aureus*, *Clostridium botulinum*, *C. perfringens* (Ryder, Karunasagar, & Ababouch, 2014). *S. aureus* (the second most common cause of food poisoning) and *C. perfringens* can produce heat-resistant toxins that can cause nausea, vomiting, abdominal cramps and, sometimes, diarrhoea (FDA, 2011; Fletcher, Boonwaat, Moore, Chavada, & Conaty, 2015).

Fungal (yeast and mould) contamination in fishery products is also a matter of concern. Species from the genera *Aspergillus*, *Penicillium* and *Achlya* have been isolated from smoked, dried and salted fish (da Silva, et al., 2008; Adeyeye, 2016; Osibona, Ogunyebi, & Samuel, 2018). These organisms are thermo-tolerant and can produce mycotoxins (e.g. aflatoxins by *Aspergillus*), which are low weight metabolites, that have public health implications for humans (Gram, 2009; Marc, et al., 2014; Adeyeye, 2016). Aflatoxins are potent carcinogens that have the potential to cause hepatoma, acute hepatitis, anemia and reduced white blood

cells, that can impair the immune system (Samaha, Amer, Abd-Elshahid, & El-bialy, 2015). *Penicillium* species can also produce mycotoxins (i.e. ochratoxin A, citrinin, patulin and penicillanic acid), that have cytotoxic effects on mammalian cell lines causing reduced cell viability (Egbuta, Mwanza, & Babalolo, 2017). These risks are however dependent on the frequency and amount (concentration) of contaminated fish consumed and the duration of exposure.

In most developing countries like Ghana, where smoked fish is sold unpacked in open-air markets, the threat of microbial contamination is very high. The unsanitary conditions of most markets, poor handling of the products by processors, traders and sometimes consumers further increase this risk. Bīlgīn, Ünlüsayın, Izci, & Günlü (2008) therefore suggest that smoked products should be stored at refrigerated temperatures (i.e. at or below 3 °C) to reduce these hazards.

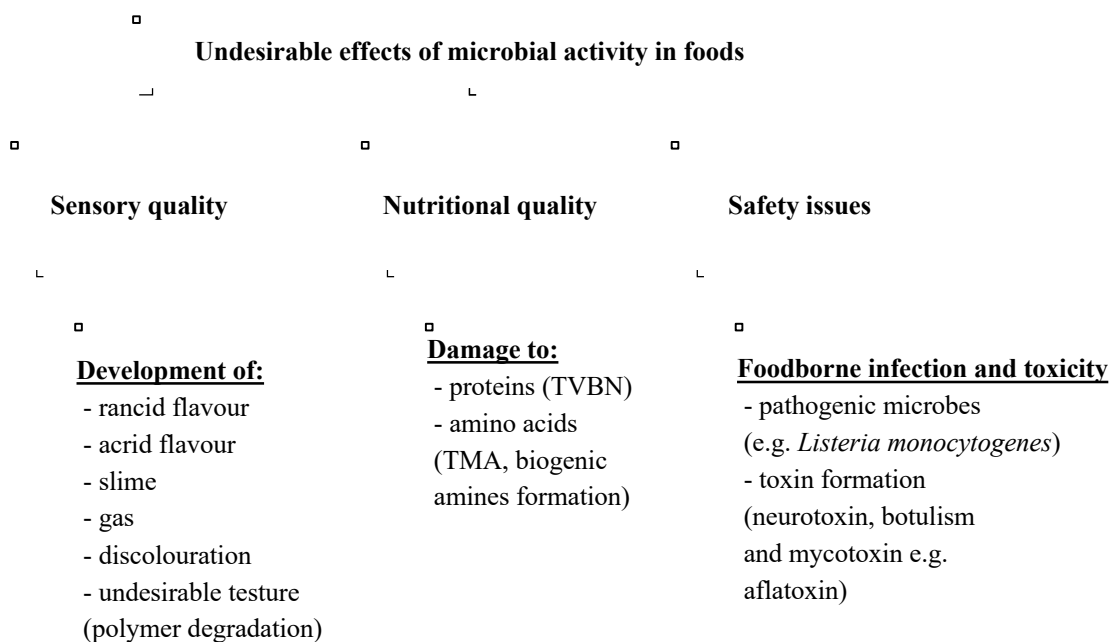


Figure 2.2: The effects of microbial activities on food quality (modified from Cyprian, 2015)

2.3.4.2 *Chemical spoilage*

One important constituent of muscle of teleosts is lipid, which are grouped as phospholipids and triglycerides (Huss, 1995). The phospholipids make up the structural lipids as they are integral in forming membranes in the cells; whereas the triglycerides are termed depot fat as they store energy, usually within special fat cells encased in phospholipid membranes (Huss, 1995). Fish lipids contain approximately 40% of long-chain fatty acids (14-22 carbon atoms), that are significantly higher than in mammalian lipids (Huss, 1995). Fish lipids are highly unsaturated and as such, can easily be oxidised or hydrolysed, throughout the processing, distribution, storage and marketing of fish (Cyprian, 2015). . In terms of human nutrition, these polyunsaturated fatty acids (PUFAs), such as linoleic and linolenic acid are very essential, as they cannot be synthesised by humans (Huss, 1995).

Oxidation can occur through non-enzymatic (autoxidation), photogenic (photooxidation) and/or enzymatic (liposygenase) reactions (Ghaly, Dave, Budge, & Brooks, 2010; Cyprian, 2015). Autoxidation of PUFA (i.e the primary cause of lipid oxidation in post mortem fish), involves free radical reactions in three stages (Figure 2.3):

- initiation: where unsaturated lipids lose a hydrogen atom to form a lipid (alkyl) radical ($L\cdot$), in the presence of an initiator;
- propagation: the alkyl radical reacts ($L\cdot$) with molecular oxygen to form peroxy radical ($LOO\cdot$), which then abstracts hydrogen from another molecule of unsaturated lipid to form hydroperoxides ($LOOH$) and a new lipid radical (L). The hydroperoxides undergoes further reaction in the presence of metal catalysts to produce secondary intermediates such as aldehydes, ketones, alcohol, small carboxylic acids and alkanes that cause off-odour, off-flavour and yellow colour development during lipid oxidation;
- and

- termination: the peroxy radicals react with each other to form non-radical products or the lipid radical reacts with an antioxidant molecule (AH) to produce inert antioxidant radicals (Huss, 1995; Kolakowska, 2003).

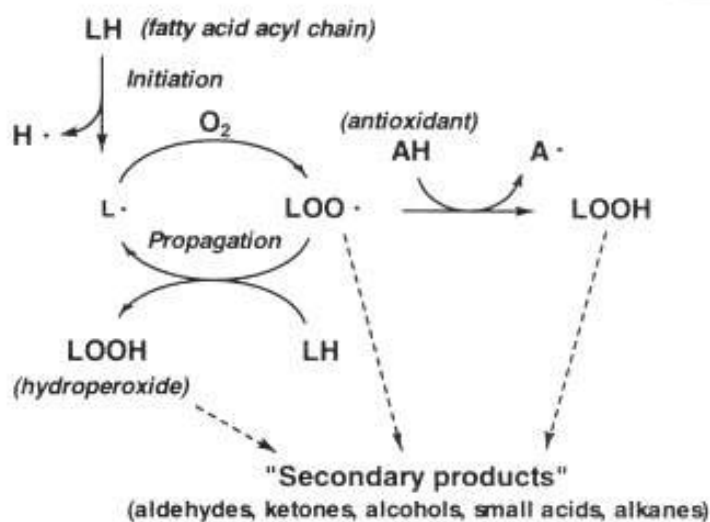


Figure 2.3: Autoxidation of polyunsaturated lipid (Adopted from Huss, 1995)

Photooxidation, on the other hand, results from a direct reaction of singlet oxygen addition to unsaturated lipids, that forms hydroperoxides but not radicals (Kolakowska, 2003). This reaction can be caused by UV light, electric shining light or irradiation (Cyprian, 2015). Lipoxygenases (enzymatic-initiate lipid oxidation) leads to the formation of hydroperoxides in a defined position of the fatty acid chain, contrary to free-radical reactions (Kolakowska & Bartosz, 2014). At the initial stage of lipid oxidation, a sudden rise in peroxide value (PV) is observed (Okpala, 2016). The extent of oxidation, however, depends on the fish species, fatty acid composition, content and action of anti- and pro-oxidants, temperature, irradiation, oxygen pressure, surface area exposed to oxygen, storage temperature and water activity (Kolakowska, 2003).

Lipid oxidation reduces the sensory and nutritive quality of fish, poses health hazards, as well as processing challenges, as summarized in Figure 2.4. Kolakowska (2003) reported that

consumption of foods rich in oxidized lipids increased thiobarbituric acid reactive substances (TBARS) in plasma and tissue of humans and animals. Aldehydes (especially malondialdehyde) can also induce intracellular oxidative stress and they are also genotoxic and react with DNA to form highly-mutagenic adducts in human cells (Reitznerová, et al., 2017).

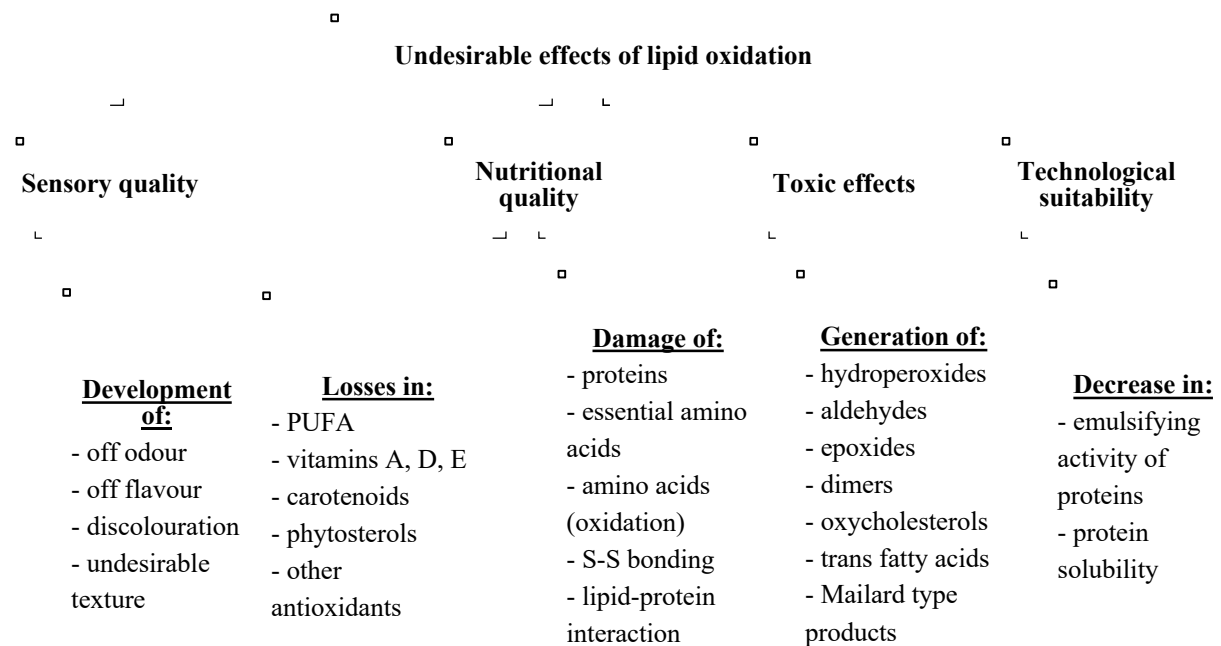


Figure 2.4: The effects of lipid oxidation on food quality (modified from Kolakowska, 2003)

Fish lipids can also be hydrolysed as a result of enzymatic activities, mainly triacyl lipase, phospholipase A2 and phospholipase B (Shah, Tokunaga, Kurihara, & Takahashi, 2009). Hydrolysis leads to the release of free fatty acids (FFAs) (Figure 2.5), which can accumulate and thus reduce the quality of the fish muscle (Kaneniwa, Yokoyama, Murata, & Kuwahara, 2004). The rate of hydrolysis depends on the fish species, temperature and also processing method, and it mostly proceeds faster in un-gutted than gutted fish perhaps due to the involvement of digestive enzymes (Huss, 1995).

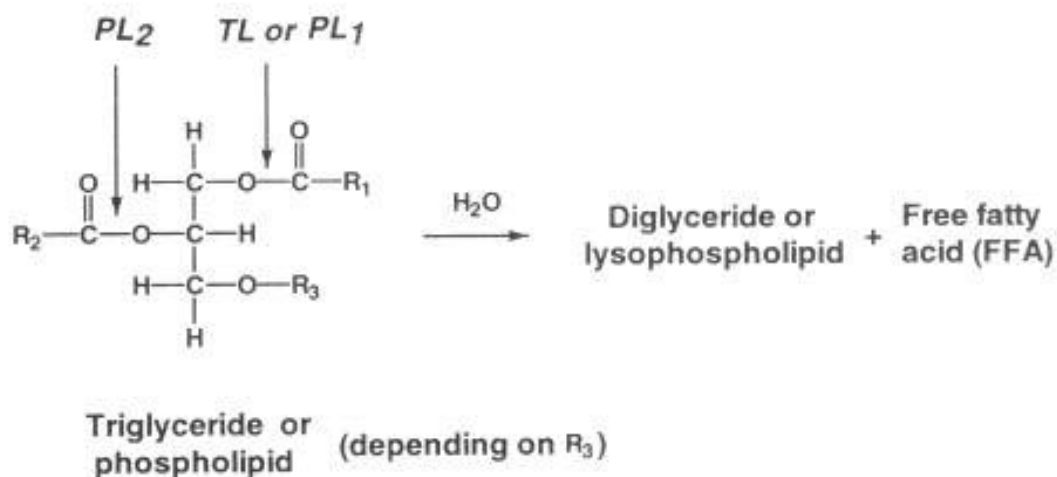


Figure 2.5: Hydrolytic reactions of triglycerides and phospholipids: PL₁ & PL₂ phospholipases; TL, triglyceride lipase (Adopted from Huss, 1995)

2.3.5 Strategies for shelf life extension of smoked fish

Fish is very sensitive to deterioration during processing, packaging, storage and marketing resulting from microbial growth. To ensure safety of the product, proper packaging and storage at temperatures that inhibit microbial proliferation are essential. Again, the products can be gamma irradiated to render them safe and shelf stable (Arvanitoyannis & Tserkezou, 2014). These strategies have been discussed below.

2.3.5.1 Packaging and storage of smoked products

Smoked fish products can be contaminated during packaging, storage and marketing (IAEA, 2001), especially where these products are sold on the open market, as happens in Ghana (Abboah-Offei, 2016). The extent of this contamination depends on the degree of smoking and drying, packaging method and temperature at which they are stored (Cyprian, 2015). It has therefore been suggested that smoked fish products must be cooled promptly at ambient or chill temperatures (immediately after smoking), and packaged ensure good quality throughout marketing (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). In Ghana, smoked fish is

usually packaged using paper materials and the products are stored at ambient temperatures, making the products susceptible to insect attacks and other forms of contamination (Kleter, 2004). Asamoah (2018) found that the use of plastic packaging materials and subsequent storage at refrigerated temperature could extend the shelf life of soft-smoked mackerel from 5 days to more than 35 days.

2.3.5.2 Irradiation

Food irradiation involves using ionising radiation, from sources such as x-rays, electron beams (e-beams) and high-energy gamma rays (cobalt-60, ^{60}Co and cesium-137, ^{137}Cs) to preserve foods (Codex Alimentarius Commission, 2003). Cobalt-60 is widely used as it offers high penetrability, permanent radiating source and high efficiency, though source replenishment is needed and low throughput is achieved (Stewart, 2004; Farkas, 2006; Arvanitoyannis, 2010). Ionising radiation is absorbed as it passes through food (in the form of invisible waves) and can reduce the incidence of food-borne illness and make food more shelf stable (WHO, 2000; Fan & Sommers, 2013). Irradiation doses are classified as low (< 1 kGy), medium (1 – 10 kGy) and high (> 10 kGy) (Arvanitoyannis, 2010). Low-dose irradiation have been shown to aid in insect/parasite disinfection, inhibit sprouting in crops such as potatoes and yam and delay ripening of fresh fruits and vegetables; medium-doses can inactivate spoilage and pathogenic microorganisms in foods and extends the shelf life and high-doses are used for industrial sterilization and decontamination of some food additives (WHO, 2000; Loaharanu, 2003; Mostafavi, Fathollahi, Motamedi, & Mirmajlessi, 2010).

To decrease/inactivate the microbiota in foods, a photon of energy or an electron collides directly with a critical element in the cell (most often DNA and RNA) or ionises an adjacent molecule (mostly water), which then reacts with the genetic material (Molins, 2001; Farkas,

2006; Mostafavi, Fathollahi, Motamedi, & Mirmajlessi, 2010). These reactions therefore prevent the multiplication of microorganisms and randomly terminates most cell functions, which improves the storability of the product (Farkas & Mohácsi-Farkas, 2011). The differences in the chemical and physical structure of microorganisms, their population and ability to recover from radiation injury will determine the success of irradiation (Farkas, 2006). Other external factors, such as moisture content, temperature during irradiation, presence or absence of oxygen, among others, also can affect the irradiation sensitivity of mostly vegetative cells (Farkas, 2006).

Irradiation can also accelerate lipid oxidation, especially in foods with high water content, since it generates hydroxyl radicals (free radicals) that are strong initiators of lipid oxidation, (Farkas, 2006; Fan & Sommers, 2013). Irradiation-induced oxidative changes are dose-dependent and can be further hastened under aerobic storage conditions (Fan & Sommers, 2013). This can affect the proteins, carbohydrates and lipids in foods; however, these are usually minor changes that are comparable to those produced from other processing technologies like pasteurisation (Ortega-Rivas, 2012). Before irradiation, the product must be properly packaged and the use of polyethylene and polypropylene bags have been suggested by (Duah, Emi-Reynolds, Kumah, & Larbi, 2018).

Fresh fish and shellfish spoil easily, as such, irradiation can be employed to improve the microbiological quality. Various studies have been evaluated the effect of ionizing radiation on the physicochemical, microbiological and sensory qualities of different seafood. Su, Duan, & Morrissey (2004) reported a log reduction of 2.5 log₁₀ cfu/g of *Listeria monocytogenes* counts in cold-smoked salmon e-beam-irradiated at 1 kGy, with a dose of 2 kGy completely eliminating the pathogen. Medina, et al. (2009) also stated that cold-smoked salmon stored at

5°C and 5°C + 8°C (temperature abuse) would require e-beam-irradiation doses of 1.5 and 3 kGy respectively to attain the Food Safety Objective (FSO) of 2 log₁₀ cfu/g for *L. monocytogenes* for a 35-day shelf-life, without affecting the visual aspect (colour) negatively. Irradiating fish at doses of 2-7 kGy and storage at refrigerated temperature reduced pathogens such as *Salmonella*, *Listeria*, and *Vibrio* spp., and many specific spoilage organisms such as *Pseudomonaceae* and *Enterobacteriaceae* (Arvanitoyannis, 2010). Al-Kahtani, et al. (1998) found no changes in amino acid in Spanish mackerel irradiated at 1.5 to 10 kGy and stored under refrigeration.

The total volatile base nitrogen levels in irradiated sea bass, tilapia and mackerel decreased during refrigerated storage, while the moisture, protein and lipid content remained unchanged for 20 days of storage (Cozzo-Siqueira, Oetterer, & Gallo, 2003; Ozden, Inugur, & Erkan, 2007). Aerobically packaged ready-to-eat smoked sardines, irradiated at 7 and 11 kGy in Ghana, were studied and the results indicated that microbial activity (i.e. aerobic counts, mould and coliforms) was controlled over 12 weeks of ambient storage, though the colour, flavour and texture were unacceptable (Nketsia-Tabiri, Adu-Gyamfi, Montford, Gbedemah, & Sefaddeh, 2003). Akuamoah, Odamtten, & Kortei (2018) irradiated dried and smoked anchovies at doses ranging from 2.5 to 10 kGy and reported shelf life increase of four weeks in irradiated samples, relative to the control group. Erkan & Ozden (2007) and Fan & Sommers (2013) however stated that the chemical properties of irradiated fish may change, depending on the species used, the initial quality at the time of capture and good management practices relating to on-board handling, icing, packaging and storage.

Studies have been conducted on the potential of gamma irradiation in improving the safety and quality of vegetables like garden eggs, mushrooms, poultry, fermented maize and cassava

products and seafood in Ghana like sardines, anchovies and shrimps (Nketsia-Tabiri, Adu-Gyamfi, Montford, Gbedemah, & Sefa-Dedeh, 2003; Adu-Gyamfi & Appiah, 2012; Adu-Gyamfi, Torgby-Tetteh, & Appiah, 2012; Adu-Gyamfi, Riverson, Afful, & Appiah, 2014; Akuamoa, Odamtten, & Kortei, 2018; Duah, Emi-Reynolds, Kumah, & Larbi, 2018). Ghana has the requisite capacity at the Radiation Technology Centre of Ghana Atomic Energy Commission, therefore, to use this technology to ensure safe and shelf-stable fish products, not only for the domestic market, but also for export (Adu-Gyamfi, Banson, & Nketsia-Tabiri, 2011).

The volumes of irradiated food products are increasing (with more than 60 countries regulating the practice), and so the future of irradiated foods looks promising (Badr, 2012; Ortega-Rivas, 2012). The FAO, World Health Organization (WHO) and International Atomic Energy Agency (IAEA) have recommended the use irradiation technology in food processing (Banson, 2015). Although there have been more than hundred years of research on irradiation, consumer perceptions have hindered its development and commercialisation (Maherani, et al., 2016). The reasons given included antinuclear activism, industry hesitation, time-consuming approval processes, and insufficient consumer education (Ehlermann, 2016). Consumer perceptions and decision to buy irradiated foods were however positively altered after they were made aware and understood the benefits of food irradiation (Nayga, Aiew, & Nichols, 2005; Mostafavi, Fathollahi, Motamedi, & Mirmajlessi, 2010) .

CHAPTER THREE

3.0 COMPARISON OF THE PERFORMANCE AND EFFICIENCY OF THE IMPROVED AND TRADITIONAL SMOKING KILNS

3.1 Introduction

Fish smoking and drying are ancient, traditional fish processing methods that are mostly employed to preserve fish in most developing countries of the world (FAO, 2018). Smoked and dried fish products have longer shelf lives and serve as the main source of fish to rural areas that are far from fishing sites (Ndiaye, Komivi, & Diei-Ouadi, 2015). In Ghana, both fresh and frozen (mostly imported) fish are smoked (Entee, 2015a). Again, the majority of fish consumed is in the smoked form (Nunoo, Asiedu, Kombat, & Samey, 2015).

Fish smoking is usually undertaken using traditional fish smoking kilns (like the metal drum and round mud kiln) and improved ones like the Chorkor, Banda, Altona, among others (Ndiaye, Komivi, & Diei-Ouadi, 2015; Bomfeh, et al., 2019). In Ghana, the most popular kiln is the Chorkor, which was developed in 1969 through the joint collaborative efforts of the Food and Agricultural Organisation (FAO) and the Food Research Institute, Ghana (Asamoah, 2018). This kiln has the advantage of being relatively more energy efficient, has greater throughput capacity, poses lower risks to processors in terms of smoke exposure and produce better quality products, compared to the other traditional kilns (Ndiaye, Komivi, & Diei-Ouadi, 2015; Entee, 2015b).

The kilns rely on solid/biomass (firewood mainly) combustion to cook, smoke and dry the fish, and this imparts the characteristic colour, odour and taste that is favoured by consumers (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014; Ndiaye, Komivi, & Diei-Ouadi, 2015; Bomfeh, et al., 2019). Biomass resources are readily accessible and provide a major source of renewable energy in most households in developing countries (Global Alliance for Clean Cookstoves, 2016). The combustion of these fuels are usually less efficient and release a variety of incomplete combustion pollutants, like carbon monoxide (CO), particulate matter (PM), sulphur dioxide (SO₂) and oxides of nitrogen (NO_x) among others, which affect human health and local/regional climate change significantly (Roy & Corscadden, 2012; Shen & Xue, 2014; Global Alliance for Clean Cookstoves, 2016). Several studies in Ghana have reported the potential public health and climate implications of using the Chorkor and other traditional fish and improved smoking kilns (Entee, 2015a, b; Flintwood-Brace, 2016; Antwi-Boasiako, 2017; Obeng, 2018; Bomfeh, et al., 2019). This calls for the development and testing of new improved and efficient fish smoking kilns.

Two improved kilns were therefore evaluated in this study and compared with the Chorkor smoker. They are both existing technologies: the Abuesi Gas Fish Smoker (AGFS) and the Cabin smoker, developed by GRATIS and UNU-FTP and Matis, respectively. The possibility of adopting these kilns will be dependent on an assessment of their performance and costs, relative to the Chorkor. The information obtained will be very useful for consumers and businesses that will be interested in these technologies.

The aim of the study was therefore to assess the performance and efficiency of the Cabin, Chorkor and AGFS smoking kilns.

3.2 Materials and methods

The efficiencies of the Cabin, Chorkor and AGFS kilns with respect to fuel usage, quantities of fish smoked, among others, were tested using the controlled cooking test (CCT) protocol. The emissions from the Chorkor and Cabin were also tested.

3.2.1 Controlled cooking test (CCT) protocol

The controlled cooking test (CCT) procedure, as described in (Bailis, 2004; Entee, 2015b; IRI-CSIR, GSA, Kwarteng, 2016) was employed to assess the efficiency of the three smoking kilns. The CCT was developed by the Global Alliance for Clean Cookstoves (Bott, 2014) to compare the performance of improved and traditional stoves, with the aim of replacing the traditional models.

3.2.1.1 Test location

The smoking experiments were carried out at the Fish Processing Facility, which belongs to the Abuesi Fish Processing Association (AFPA), at Abuesi in the Western Region of Ghana from 12th to 14th July 2018. Abuesi is about 20 km from Takoradi, the Western Regional capital. Abuesi has a population of about 10,000, with about 52% being females. The main economic activities are fishing and trading of fish, particularly, smoked fish (Ghana Statistical Service, 2014). Fish smoking is mainly carried out on small scale basis in traditional smokehouses, which could be enclosed or open (Flintwood–Brace, 2016). This site was selected because of the availability of the Abuesi Gas Fish Smoker and a prototype of the Cabin kiln. Again, Abuesi was selected because of the high usage of the traditional fish smoking kilns and the reported respiratory risks observed in fish processors (Flintwood–Brace, 2016).

3.2.1.2 Description of fish smoking kilns tested

The Chorkor, Cabin and AGFS kilns, representing uncontrolled and semi-controlled and controlled smoking technologies respectively, were used in the smoking experiment. The Chorkor and Cabin kilns use firewood to smoke the fish, while the AGFS used LPG gas.

Chorkor Kiln

The Chorkor kiln (Plate 3.1) is built with blocks. For the purpose of this CCT, only one side of the kiln was used and described accordingly. The kiln dimensions are 120 x 150 x 59 cm (L x B x H) with wall width of 13 cm. The kiln can hold between eight (8) to twelve (12) wooden trays, with an estimated maximum capacity of 120 kg. The tray measurements are 109 x 98 x 5 cm (L x B x H), with a wire mesh area of 9,476 cm² (0.95 m²). There is one (1) front loading firepot, with dimensions of 94 x 78 x 80 cm (L x B x H). The trays are loaded atop the fireplace with no locking mechanism. The ends of the trays are fashioned into handles for easy handling.



Plate 3.1: Chorkor smoking kiln

Cabin kiln

The Cabin (Plate 3.2) is built with fired bricks. It has an overall dimension of 140 x 138 x 187 cm (L x B x H), with a brick wall width of 12.5 cm. The kiln has seven (7) wooden trays that

are arranged on wooden rails inside the smoking chamber, with an estimated maximum capacity of 90 kg of fish. Each tray has dimensions of 112 x 104 x 7 cm (L x B x H), with a wire mesh surface area of 10,388 cm² (1.04 m²). The kiln has a front loading firepot with dimension 77 x 65 x 65 cm (L x B x H). This firepot is covered by a curved metal plate of dimensions 100 x 90 cm (L x H). There is a metal door of dimension 73 x 70 cm (L x H) that seals the firepot. The distance between the steel plate and the first tray is 38 cm. There are two ventilation holes on either side of the kiln with dimensions, 26 x 12.5 x 10 cm (L x B x H). The kiln has an opening at the top that serves as a conduit for smoke release.



Plate 3.2: Cabin kiln

Abuesi gas fish smoker (AGFS)

The AGFS (Plate 3.3) is an industrial, double-chamber fish smoking oven built from stainless steel. It is a double-chamber oven but for the purposes of the study, only one chamber will be

described. The dimensions of one chamber measure 120 x 180 x 240 cm (L x B x H). Twenty five metal trays arranged on rails can be used per smoking section but for proper aeration, the recommended number of trays is 15 to 18. The estimated maximum capacity is 500 kg of fresh fish. Each tray has dimensions of 116 x 168 cm (L x B), with a wire mesh surface area of 18,800 cm² (1.88 m²). Below the bottom tray the perforated metal burner, which is connected via hose to an LPG gas cylinder. The chamber has a suction fan (operated by electricity) that extracts moisture from the oven during the drying stage of the smoking process. There is a chimney that goes through the roof of the building. There is a smoked generator (50 x 10 x 70 cm) attached to the side of the chamber with a hole connecting the two. Agricultural by-products e.g. sugarcane bagasse and coconut husk are burnt in this unit to provide the smoked flavour.



Plate 3.3: The Abuesi gas fish smoker (AGFS)

3.2.1.3 Fuel used

Bundles of the firewood (Plate 3.4), 'Kontan' (*Uapaca guineensis*), were obtained from local sellers at Abuesi in the Western Region. This is a hardwood with a calorific value of 4,126 Kcal/kg (Last, Richards, & Fyfe, 1987). The mean length and width of 54 x 11.6 cm was estimated from measuring 16 pieces of firewood sampled from the bundles used. The moisture content of the wood was also measured at three different locations in each wood, for 20 samples using the General Tools MMD4E Moisture Meter. The mean moisture content was then estimated as 19.2%.



(a)

(b)

Plate 3.4: 'Kontan' (*Uapaca guineensis*) used in smoking trials (a) and measuring wood moisture (b) for the CCT trials

In the AGFS, the cooking and smoking step was accomplished using LPG gas and sugarcane and then the dryer (which uses electricity) was turned afterwards.

3.2.2 Atmospheric conditions

The temperature and humidity were measured during the testing period. The mean temperature and relative humidity were 25.7°C and 85.7% respectively. There was a light breeze during that time.

3.2.3 Experimental procedures

Two local fish processors, each with with more than 10 years fish smoking experience, who use smoking kilns were recruited for the CCT, under the supervision of the researcher. Each processor tested each kiln once daily, over the three-day period. Each processor was given 60 kg of fish per kiln (Chorkor and Cabin) per day and 60 kg of firewood per kiln per day (which was about twice the quantity of the fuel that they estimated would be enough to smoke all the fish per day). The smoking was accomplished in two steps: a cooking and smoking step and a drying step. The weights of fish and wood were taken using a KERN CH50K100 electronic, hanging balance (KERN & Sohn GmbH, Germany), with a maximum capacity of 50 kg and a resolution of 0.10 kg. For the AFGS, 100 kg of fish was used for each smoking session due to its industrial size and also to avoid excessive fuel wastage. During the drying step, the oven fan was turned on for about 30 minutes to circulate the hot air, after which it was turned off and the residual heat used for further drying. The processor was given a 30 kg LPG cylinder per smoking session. The temperature inside the fish was taken at 30-minute intervals with a Thermco Precision Handheld Pt100 Digital Thermometer (resolution of 0.1 °C). Once the temperature inside the fish reached 70 °C (temperature threshold to kill bacteria on the skin and in the muscle), the fish were allowed to be in the kiln for an extra 30 minutes, as described in Asamoah (2018), after which they were considered smoked and taken out.

3.2.3.1 Fish samples preparation

Frozen chub mackerel (*Scomber colias*), procured from cold stores, was used in the smoking trials. The fish was thawed at ambient temperature for about 2 hours and then weighed. After weighing, the fish was washed and then brined in an 8% NaCl solution for 30 minutes. They were taken out of the brine solution, arranged on the smoking racks and allowed to dry for about 30 minutes before smoking started.

3.2.3.2 *Stack Emissions Monitoring*

The carbon monoxide (CO), sulphur dioxide (SO₂) oxides of nitrogen (NO_x) and particulate matter (PM) emissions were measured for the Cabin and Chorkor kilns using the E9000 Portable Stack Emissions Analyser. The probe of the analyser was inserted into the smoke ‘escape’ hole on top of the Cabin and in the fireplace of the Chorkor kiln, as shown in Plate 3.5. The gas sample was drawn through the probe, with a diaphragm suction pump. The gas sample was then cleaned of humidity and impurities by a condensate trap and filter located inside the instrument. The gas components were analysed using the electrochemical and infrared sensors. The CO, SO₂ and NO_x were tested following the BS EN 15058, TGN M21 and BS EN14794 methods.

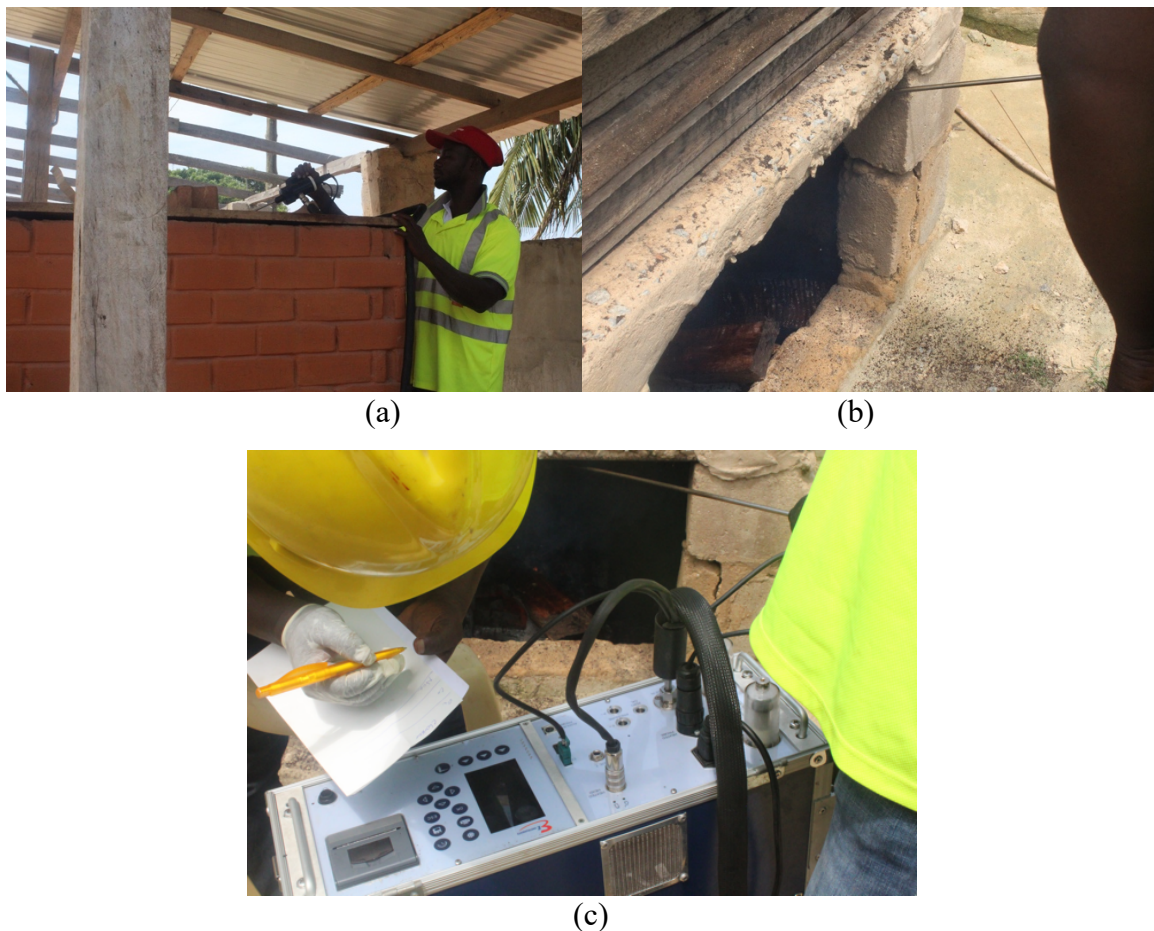


Plate 3.5: Stack emissions monitoring of (a) Cabin and (b) Chorkor kilns; (c) E9000 emission system

The particulate matter measurement was done using the RESS stack pump, a component of the E9000 emission system (Plate 3.6). PM was measured isokinetically (BS EN 13284-1 method) by placing a previously conditioned glass micro fibre filter in the RESS stack pump. The pump was then inserted into the same place as used for the gas emissions. The flue gas was drawn at a flow rate of 1.9 LPM, in ten uniform pulls per kiln. After this, the filter paper was removed and kept in the zip lock bag for laboratory analysis. In the laboratory, the mass of the deposited particles was analysed to obtain the mass per unit volume of PM particles present in the emission. The emission tests were done for 1 hour. For quality assurance and control purposes for the emissions monitoring, all equipment were calibrated with span gases, prior to the measurements, to authenticate the precision of the equipment and data generated. On the site the auto zero calibration phase was also activated, then compared with the programmed values and compensated for standard deviations. All filters for particulate matter were dried, weighed and kept in silica gel to keep the integrity of the filter before measurement.



(a)

(b)

Plate 3.6: PM monitoring (a) in Cabin kiln and filter paper showing PM residue (b) for analysis

3.2.4 Smoked products measurements

After smoking, the fish from each kiln was cooled at room temperature (about 26°C) and then weighed. Plate 3.7 depicts the smoked products from the three kilns. To determine the moisture contents of the smoked fish, 5 g of the fish sample was dehydrated at 105°C for 4 hours to constant weight (AOAC, 2005). The loss in weight during drying was expressed as g water/100 g sample.

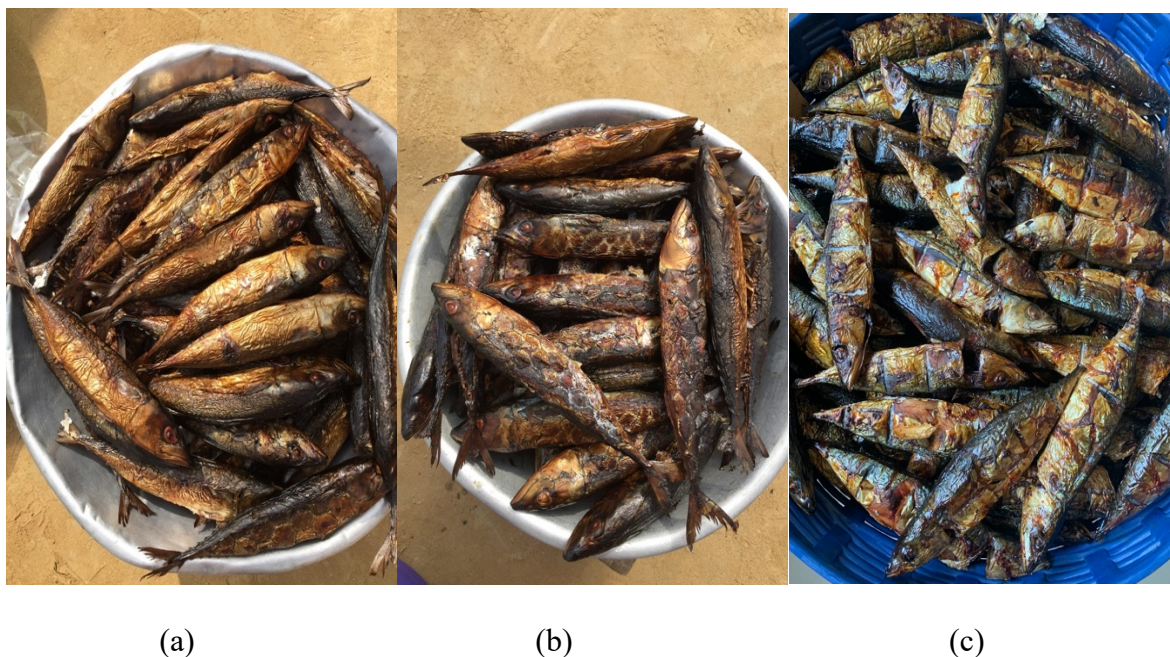


Plate 3.7: Fish sample from the (a) Cabin, (b) Chorkor and (c) AGFS smoking kilns

3.2.5 Data and Statistical Analysis

Statistical analysis was performed using Microsoft Excel 2016 and XLSTAT (Addinsoft, New York, USA). The data from the CCTs were inputted into the CCT 2.0 Spreadsheet software (Global Alliance for Clean Cookstoves, 2018). The software estimated the equivalent dry wood consumed (kg), specific fuel consumption (kg of firewood used per kg of fish smoked) and total cooking time (min), for the individual tests, based on the equations below:

$$\text{Equivalent dry wood consumed } (f_d) = (f_i - f_f) * (1 - (1.12 * m)) - 1.5 * \Delta C_c \quad \text{Equation 3.1}$$

$$\text{Specific fuel consumption } (SC) = \frac{f_d}{W_f} * 1000 \quad \text{Equation 3.2}$$

$$\text{Total smoking time } (\Delta t) = t_f - t_i \quad \text{Equation 3.3}$$

Where: f_i and f_f are the initial and final weights of firewood respectively (g); m is the wood moisture content (%); ΔC_c is the weight of char remaining (g); W_f is the weight of smoked fish (g); and t_i and t_f are the start and finish times of smoking (min) (Bailis, 2004).

The mean of the three tests performed per stove was then estimated. The results were tested for statistical significance using one-way ANOVA followed by Tukey's HSD.

The smoking yield (%) was also calculated according to (Cyprian, et al., 2015) as:

$$\text{Smoking yield } (\%) = \left(\frac{W_s}{W_r} \right) * 100 \quad \text{Equation 3.4}$$

Where W_s is the weight of smoked fish and W_r is the weight of raw fish.

Also, the processing rate (g/min), cost of firewood used per kiln (GHS) and the cost of firewood per kg of smoked fish (GHS/kg) were estimated. The conversion rate, as of 3rd June 2019 was USD 1 = GHS 5.20 (Bank of Ghana, 2019). For the AGFS, similar estimates were made but some costs like that for electricity could not be estimated. Results are presented as mean \pm standard deviation.

3.3 Results

3.3.1 Efficiency of the smoking kilns

The moisture contents of the smoked mackerel were 38.87, 33.71 and 25.38 g/100g for the Chorkor, Cabin and AGFS kilns respectively. The results from the CCT are presented in Table 3.1. The mean total weights of smoked fish were 34.6, 34.1 and 50.2 kg, which corresponded to yields of 57.6, 56.9% and 50.2% for the Chorkor, Cabin and AGFS kilns respectively. The AGFS had significantly lower ($p < 0.05$) yields compared to the Chorkor and Cabin. The equivalent dry wood consumed in the Chorkor averaged 33.2 kg compared to the Cabin which used 23.3 kg. This represented a 30% lower usage in the Cabin compared to the Chorkor, and this was statistically significant ($p < 0.05$). The mean quantity of gas and sugarcane consumed were 15.3 and 0.3 kg respectively per smoking trial, which in total was significantly lower ($p < 0.05$) compared to the Chorkor and cabin kilns. The mean specific fuel consumption per kg of smoked fish in the Chorkor, Cabin and AGFS were 0.96, 0.67 and 0.31, which were all statistically different ($p < 0.05$). The amount of char remaining in the Chorkor averaged 0.35 kg, which was significantly lower ($p < 0.05$) than in the Cabin, which produced 0.99 kg of char.

The total smoking time was recorded when the racks were placed on/in the kilns till they were taken out for weighing. The Chorkor used a mean of 256 minutes while the Cabin used 218 minutes, representing a 15% less time to smoke that was statistically significant ($p < 0.05$). The AGFS also used 200 minutes, which was 22% significantly lower ($p < 0.05$) than the Chorkor. The processing rate of the Chorkor was 135.2 g/min, which was about 16% significantly lower than ($p < 0.05$) the Cabin, with 156.8 g/min rate. That for AGFS was 251 g/min, which was 86% and 60% significantly higher ($p < 0.05$) than the Chorkor and Cabin respectively.

The cost of firewood per kg was estimated at GHS 0.84. The mean cost of firewood by the Chorkor and Cabin kilns were GHS 36.09 and 28.76 respectively per smoking session. There was a fuel saving of GHS 7.34, on average when the Cabin was used, compared to the Chorkor and this was statistically significant ($p < 0.05$). The cost of firewood/kg of smoked fish was also significantly higher ($p < 0.05$) in the Chorkor compared to the Cabin (i.e. GHS1.05 and 0.78/kg for Chorkor and Cabin respectively). The mean cost of gas used was GHS 76.33 and it cost GHS 1.59 to produce 1 kg of smoked fish, which was significantly higher ($p < 0.05$) than when using firewood. The cost of electricity used for drying the fish could however not be determined, and this could increase the cost. The cost of sugarcane used for the smoked flavoring was about GHS 0.10, which was very low.

Table 3.1: Comparison of efficiency of Chorkor, Cabin and AGFS kilns (Mean \pm SD)

Parameter	Chorkor	Cabin	AGFS
Weight of fresh fish (kg)	60.00 \pm 0.00	60.00 \pm 0.00	100.0 \pm 0.0
Weight of smoked fish (kg)	34.57 \pm 1.07	34.13 \pm 0.49	50.17 \pm 0.76
Yield (%)	57.61 \pm 1.80 ^a	56.89 \pm 0.80 ^a	50.17 \pm 0.76 ^b
Weight of char remaining (kg)	0.35 \pm 0.35 ^a	0.99 \pm 0.16 ^b	-
Equivalent fuel consumed (kg)	33.20 \pm 3.39 ^a	23.29 \pm 0.46 ^b	15.60 \pm 0.92 ^c
Specific fuel consumption /kg of smoked fish	0.96 \pm 0.12 ^a	0.67 \pm 0.01 ^b	0.31 \pm 0.02 ^c
Total smoking time (min)	256 \pm 15 ^a	218 \pm 9 ^b	200 \pm 10 ^b
Processing rate (g/min)	135.20 \pm 5.60 ^a	156.80 \pm 8.30 ^b	251.18 \pm 10.65 ^c
Cost of fuel/kg of smoked fish (GHS/kg)	1.05 \pm 0.12 ^a	0.78 \pm 0.01 ^a	1.69 \pm 0.17 ^b
Maximum capacity (kg)	120.0	90.0	500
Construction cost (GHS)	950.00	4,350.00	65,000.00

^{a, b, c} Means with different superscripts within a row are significantly different at $\alpha < 0.05$.

The cost of construction was GHS 4,350.00, GHS 950.00 and GHS 60,000 for the Cabin, Chorkor and AGFS respectively. The major costs items for the Cabin were those of the steel

door and plate (28%) and bricks (25%), whereas that for the AGFS were the aluminum frame and the capacity. The estimated lifespan of the Cabin and Chorkor is about 10 years, but the smoking racks may last for between 2 to 4 years. The AGFS can also last for about 15 years.

3.3.2 Emissions test

The results from the stack emissions tests are presented in Table 3.2. The Chorkor kiln recorded mean emission values of 2058.5, 33.5, 19.25 and 400 mg/m³ for CO, NO₂, SO₂ and PM respectively. The cabin kiln also recorded 1792.4, 30.0, 4.5 and 398.2 mg/m³ for CO, NO₂, SO₂ and PM respectively. These emissions were not statistically different ($p > 0.05$), with the exception of SO₂. The flue temperatures for the Chorkor and Cabin were 136.6 and 131.1°C respectively.

During the smoking trials, it was observed that there were periods of high smoke production in the Cabin at the start of the smoking trial and during addition of extra wood. Once the wood burnt to embers, smoke production was considerably reduced. The Chorkor behaved similarly, except that there was continual but reduced smoked production as the test progressed. The continued smoking was as a result of the fish oils dropping into the fire directly.

Table 3.2: Comparison of emissions (mg/m³) from the Chorkor and Cabin kilns (Mean ± SD)

Parameters	Chorkor	Cabin
CO	2058.5±180.3	1792.3±13.1
NO _x	33.5±2.1	30.0±7.1
SO ₂	19.3±7.4 ^a	4.5±6.36 ^b
PM	400.0±10.0	398.2±1.9

CO is carbon monoxide, NO_x is oxides of nitrogen, SO₂ is sulphur dioxide and PM is particulate matter

3.4 Discussion

The efficiency of a kiln is measured by the temperature distribution within the kiln, the specific fuel consumption, the throughput capacity, the length of time spent smoking and the quality of the final product, in comparison with another (Hilderbrand, 1992; Bailis, 2004; Entee, 2015b). With respect to the throughput capacity, it was estimated that the Cabin and Chorkor could each load approximately 90 and 120 kg respectively per smoking session. From the CCT, the Cabin and Chorkor kilns used 683.4 g and 955.1 g of firewood respectively per kg of smoked fish. The Cabin was thus more fuel efficient, saving about 29% more firewood, which was significantly lower compared to the Chorkor. This saving potential did not however meet the 40% fuel saving requirement of Energising Development (EnDev) programme (Entee, 2015b). According to the processors, the remaining charcoal (char) was not used in subsequent fish smoking but used to fuel their local stoves in the preparation of household meals. This was an added benefit to them.

A study by IRI-CSIR, GSA, Kwarteng (2016), which compared the Chorkor and Ahotor kilns in Ghana, gave the Ahotor kiln a fuel saving potential of 32% over the Chorkor kiln. Ahotor recorded higher fuel saving than that recorded by the Cabin in this study. The higher potential of the Ahotor could however be attributed to the fact that the moisture content of the firewood was high (61.1%) compared to that from this study which was 19.2%. Taylor (2010) found that firewood with moisture content of 60% and above burn slowly and can lead to incomplete combustion, that produces dirty smoke, which is not healthy. The best firewood for effective burning must therefore be properly seasoned/dried and must have a moisture content below 20% (Taylor, 2010). Dry firewood has been shown to burn hotter and completely, as less heat is used up evaporating water, guaranteeing a cleaner and safer fire (Taylor, 2010).

The time spent smoking in the Cabin was 15% less and this corresponded to a higher processing rate (16%) compared to the Chorkor. The closed design of the Cabin and the control of air entering the fireplace may have contributed to its fuel and time savings as well as higher processing rate, as most of the heat was kept in the smoke chamber and the fire burned in a controlled way. The smoking time was less than that in a similar study by Entee (2015b), who reported smoking times of 285 to 709 minutes (corresponding to processing rates of 190.4 and 27.5 g/min) in the Morrison and Association of Women for the Preservation of the Environment (AWEP) kilns respectively. That for Chorkor was higher than in the present study but the FAO Thiaroye Technique (FTT) had a rate of 44.2 g/min.

A comparison of the Chorkor and Cabin kilns to the AGFS showed that the latter performed best, followed by the Cabin and then the Chorkor kilns. In terms throughput capacity, the latter could hold more fish per smoking cycle (maximum capacity of 500 kg) and smoked it faster than the former. Thus, the processing rate was much faster. This could be very useful, especially during the bumper fish season, in reducing the postharvest losses that are encountered (Akande & Diei-Ouadi, 2010). The AGFS had a fuel saving potential of 68% and 54% higher than the Chorkor and Cabin respectively, which met the 40% fuel saving requirement of EnDev (Entee, 2015b). The yield was however significantly lower in the AGFS and was as a result of the superior drying achieved. This, combined with the reduced moisture content, could make products more shelf stable, relative to those from the Chorkor and Cabin (Asamoah, 2018). Yield, on the other hand, is also of high economic importance as the price of smoked fish mostly depends on the weight (Entee, 2015a) so this might affect the profitability of the operation. The cost of fuel was higher than when firewood was used, and this is as a result of the high price of LPG generally (about GHS 5 per kg compared to wood which was GHS 0.84 per kg in this study). However, with about 51% Ghanaians dependent on

firewood and another 35% relying on charcoal (derived from wood), as their primary fuel source, coupled with the fact that about 72% of the country is vulnerable to desertification, firewood is considered an unsustainable choice (Global Alliance for Clean Cookstoves, 2016).

In terms of construction costs of the kilns, the Chorkor was the lowest with the AGFS being the highest. A study comparing the costs of fish smoking kiln in Ghana estimated the costs of approximately GHS 1,200.00 and 5,600.00 for the Chorkor and FTT kilns respectively (Entee, 2015b). The AGFS is an industrial kiln but the cost might be a deterrent for small scale processors. There are however other sizes and specifications that costs less, and processors can explore these options. Further, the fear of accidental explosions of the gas plus sometimes unreliable supply of gas in the country might be further barriers for its adoption (Nunoo, Asiedu, Kombat, & Samey, 2015).

From the results, with the exception of SO₂, the concentrations of CO, NO_x and PM were similar between the two kilns. The incomplete combustion of biomass fuels like firewood as a result of low temperature of combustion, inadequate oxygen and poor mixing of fuel and combustion air may have resulted in the high concentrations of CO released, as suggested by Xiu, et al. (2018). A comparison of different biomass fuels in domestic wood stoves produced between 750 to above 3000 mg/m³ and this was attributed to limited mixing of air with volatiles in the biomass fuel (Roy & Corscadden, 2012).

Oxides of nitrogen (NO_x) may be formed from a mixture of atmospheric nitrogen (that forms thermal NO_x above 1300°C temperatures), prompt NO_x (formed at the flame front) and elemental nitrogen content in the fuel (which forms fuel-NO_x on combustion) (Roy & Corscadden, 2012; Russell, 2013). With biomass burning at temperatures below 1300°C, the

level of NO_x is usually low as it is formed from the nitrogen constituents in the fuel (Roy & Corscadden, 2012; Trozzi, 2013; Rabaçal, 2013; Xiu, et al., 2018). This may have accounted for the low levels obtained in this study. The elemental sulphur in wood during combustion is mostly converted to sulphur dioxide (SO_2), with the levels directly correlating to the amount of fuel used (Zandaryaa & Buekens, 2009; Russell, 2013). This assertion agrees with the results from this study that showed a significantly lower SO_2 level in the Cabin which correlated with its lower fuel consumption, relative to the Chorkor kiln.

The combustion of biomass fuels can further result in the formation of particulate matter (PM), whose main constituents are soot and condensed and adsorbed organic vapour (Sippula, 2009; Garcia, Borrega, & Luís, 2018). Roy & Corscadden (2012) reported PM emission was dependent on the type of fuel used, with hardwood having lower emissions than softwood. For environmental and human health protections, Ghana's Environmental Protection Agency (EPA) has set standards for point source/stack emissions. Per the guidelines, the limits for CO, SO_2 , NO_x and PM are 100, 200, 200 and 50 mg/m^3 respectively (EPA, 2019). Comparing the results obtained in this study to the EPA limits indicated that SO_2 and NO_x were below, whereas CO and PM were about eighteen and eight times higher than the set limits respectively. The high levels of CO and PM could be because there were no pollution reduction mechanisms incorporated into the design of the kiln, which the EPA (2019) expected.

The high levels of CO and PM could negatively impact the health of processors and the community as a whole, since emissions can travel long distances in the atmosphere and sometimes form secondary products in the atmosphere (Edwards, 2014). Particulate matter emissions can negatively affect the respiratory systems, damage lung tissue, cause cancer, and ultimately premature deaths in especially children, elderly and asthmatics (Global Alliance for Clean Cookstoves, 2016; Garcia, Borrega, & Luís, 2018). Some studies have suggested the

adoption of fish smoking kilns that utilise charcoal since they posed little environmental risk compared to the Chorkor and other kilns that use firewood (Entee, 2015a, b; Ndiaye, Komivi, & Diei Ouadi, 2015; Bomfeh, et al., 2019). It was however reported that the production of charcoal alone had an estimated 2.4 times PM formation impact than firewood and LPG used in cookstoves in Ghana (Global Alliance for Clean Cookstoves, 2016).

It was assumed that closed nature of the Cabin and AGFS (with vents on top to direct smoke) as well as the shorter time spent smoking, as compared to the Chorkor, might reduce the emissions that the processors will be exposed to. There have been some recent studies in Ghana that have investigated personal exposure of processors and non-processor to CO and PM emissions in fish smoking areas (Antwi-Boasiako, 2017; Obeng, 2018). These studies reported PM emissions higher than the EPA standards, even in control locations that were further away from area where fish smoking took place. This suggested there was some communal pollution from fish smoking that was attributed to the siting of the facilities in the study areas. CO on the other hand was below the guideline values prescribed by the EPA. Carbon monoxide is however lethal, even at minimal exposure levels, and so continuous exposure may result in headaches, nausea, convulsions, increased heart rate, unconsciousness and death (at high concentration), especially to vulnerable population groups (Goldstein, 2008; Lucarelli, Wyne, & Svenson, 2018; Bilsback, et al., 2019).

3.5 Summary of findings

Three kilns, the Chorkor, Cabin and AGFS kilns representing uncontrolled, semi-controlled and controlled smoking technologies, were investigated based on their efficiency and amount of flue gases produced. The yield after smoking followed the trend of the AGFS < Cabin < Chorkor and conversely, the specific fuel consumption/kg of smoked fish and processing rate

followed the trend of Chorkor < Cabin < AGFS. These findings were most likely generated because of the higher heat loss to the surroundings in the case of the Chorkor kiln compared to the two. This led to an uneven distribution of heat in the Chorkor kiln which resulted in higher consumption of fuel, longer processing times and higher yield. Furthermore, the kilns which used firewood as fuel (Chorkor and Cabin) produced flue gases containing CO and PM were that well above Ghana's EPA emissions standard. For SO₂, NO_x however, the amount produced was below the emission standard and therefore it stands to reason that with better heat management, the firewood consumption, CO and PM emission could be decreased. This would help safeguard the health of the predominately female population who use these kilns and their environments. To conclude, the AGFS kiln showed a better performance than the Chorkor and Cabin but due to fuel and cost consideration, the Cabin kiln has the flexibility to be suited to the fishing communities in Ghana in the short run. The environmental benefits associated with the AGFS and its industrial operations however make it a preferable choice, in the long run.

CHAPTER FOUR

4.0 ASSESSMENT OF THE QUALITY OF TWO COMMERCIALY IMPORTANT FISH SPECIES SMOKED USING TWO DIFFERENT KILNS IN GHANA

4.1 Introduction

Fish and fisheries products provide essential amino acids, highly polyunsaturated fatty acids (PUFAs) like omega-3 and omega-6, vitamins (A, B₂, and D) and minerals (calcium, phosphorus, iodine, iron, zinc, magnesium, selenium and potassium), all of which are essential for healthy living (Hyldig, Larsen, & Green-Petersen, 2007; Stadlmayr, et al., 2010; Pal et al., 2018). In Ghana, about 75% of the annual national fish production is locally consumed, which accounts for about 50% of the animal protein intake (MOFA, 2017; FAO, 2018). As a highly perishable commodity, fish requires some degree of processing. This is because it begins to deteriorate immediately after harvest. According to FAO (2016a), the processing of fish preserves and extends its shelf life and also allows for extended distribution and marketing opportunities.

Fish smoking is an ancient, traditional and affordable preservation method used in most parts of the world, especially in areas where there are logistical challenges in cold storage (Asamoah, 2018). Smoking enhances qualities of great consumer demand like flavour, colour and texture (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). It also prolongs the shelf life of the product due to the combined effects of salting, dehydration and antimicrobial and antioxidant activity of some smoke constituents (Doe, 1998; Goulas & Kontominas, 2005; Codex Alimentarius, 2013). In Ghana, it has been estimated that about 70-80% of fish consumed is smoked (Nunoo, Asiedu, Kombat, & Samey, 2015), with the products used in soups and sauces

(UNDP/TCDC, 2001). The mackerel, sardines, anchovies and barracuda are the main smoked fish products of commercial importance in Ghana (Nunoo, Asiedu, Kombat, & Samey, 2015).

There have been concerns about the quality and safety of fresh and smoked fish in the country. Healthy, freshly caught fish have sterile muscles but the surfaces of the skin, gills and gastrointestinal tracts could harbour microorganisms (Gram, 2009). These microorganisms could cause spoilage during processing and subsequent storage and display at the various markets (Plahar, Nerquaye-Tetteh, & Annan, 1999; Edris, Hasanen, Khater, & Lela, 2012; Bomfeh, 2016; Aheto, et al., 2017). In terms of safety, microbial contamination has been strongly linked to cases of food-borne infections like cholera, salmonellosis, staphylococcal intoxication, histamine poisoning, among others (ICMSF, 1986; FDA, 2011; Visciano, Schirone, Tofalo, & Suzzi, 2012; Berjia & Brimer, 2013; Costa, 2013). In addition, there are chemical and enzymatic changes, and all these factors can affect the colour, flavour, texture and odour, which are important sensory attributes that can influence consumer acceptance (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). They can also affect the nutritional status of the processed fish by damaging proteins and amino acids (Cyprian, 2015).

The raw material quality, type of smoking technology used, composition of smoke, among others, can affect the quality of smoked fish (Doe, 1998; Onyia, Sogbesan, Milam, & Joseph, 2011; Sogbesan & Ibrahim, 2017). This study therefore sought to investigate the quality of fresh Atlantic chub mackerel (*S. colias*) and the European barracuda (*S. sphyraena*) and the possible influence of smoking on the quality of the smoked products. It is also aimed at assessing the physicochemical, microbial and sensory qualities of the smoked products from two different fish smoking kilns.

4.2 Materials and Methods

4.2.1 Description of study area

The smoking experiments were carried out, on fish purchased in January and July 2018, at the Fish Processing Facility, which belongs to the Abuesi Fish Processing Association (AFPA), at Abuesi in the Western Region of Ghana (described in Chapter Three).

4.2.2 Fish smoking kilns

Smoking was carried out using two different smoking kilns, i.e. the Abuesi Gas Fish Smoker (AGFS) and the Cabin kiln (Chapter Three). The AGFS and Cabin represented controlled and semi-controlled smoking technologies. The AGFS operates on liquefied petroleum gas (LPG), for heating and cooking and agricultural products like sugarcane (cut pieces or bagasse) for smoke generation. It is constructed from steel with removable metal smoking racks. The Cabin on the other hand relies on firewood as the smoking fuel and it is constructed from burnt bricks with wooden doors and removable wooden smoking racks.

4.2.3 Fish sample acquisition and processing

Two batches of Atlantic chub mackerel (*Scomber colias*, Gmelin, 1789) and the European barracuda (*Sphyraena sphyraena*, Linnaeus, 1758), landed on 19th January 2018 and 13th July 2018, were purchased from artisanal fishers in Sekondi (in the Western Region) for the assessment. Twenty kilograms (20 kg) each of mackerel and barracuda were gutted and subjected to brining, by immersion in an 8% brine solution (fish: brine ratio of 1: 2 w/v) for 30 minutes. After brining, the samples were placed on racks to drain for 30 minutes before smoking. The mackerel was smoked whole, whereas the barracuda was smoked coiled as pertains to how the fish is prepared by local fish processors. The fish samples were smoked for

approximately 4 hours in both kilns. Fish samples were taken out and turned upside down midway during the smoking period to ensure even smoking. After smoking, the fish were cooled at ambient temperature for about an hour, after which they were packed into ziplock bags, iced and transported to the laboratory for analysis.

4.2.4 Analyses

The physicochemical, microbial and sensory quality of the samples were assessed, in duplicate, for fresh and smoked fish. Laboratory analyses were conducted at the Food Microbiology and Sensory Laboratories of the Department of Nutrition and Food Science, University of Ghana and the Food Research Institute of the Centre for Scientific and Industrial Research (FRI-CSIR).

4.2.4.1 Physical analysis

Condition factor

The total length and weight measurements of 30 individual fresh fish samples of each species were taken. The condition factor (K; g/cm³) was calculated for each fish species using the Fulton's index (Froese, 2006) as:

$$K = 100W/L^3 \quad \text{Equation 4.1}$$

where W is the ungutted weight of fish (g) and L is the length of fish (cm).

Colour Measurements

The skin and muscle colour of smoked mackerel and barracuda were measured with a Minolta CR-310 chromameter (Minolta Camera Co., Ltd; Osaka, Japan). The chromameter was calibrated with a reference white porcelain tile ($L = 97.57$, $a = +2.29$, and $b = 1.88$) before

measurements. The colour intensity was described in L^* , a^* , and b^* notation on the CIE LAB colour scale, according to (CIE, 1979). The L^* measured lightness ($L^* = 0$ for black and $L^* = 100$ for white); a^* defined components on the red-green axis ($a^* > 0$ for red and $a^* < 0$ for green), and b^* defined components on the yellow-blue axis ($b^* > 0$ for yellow and $b^* < 0$ blue) (Cardinal, et al., 2004). Measurements were made at three locations from posterior to anterior, on the skin surface and muscle of the smoked fish samples and the mean and standard deviation calculated. The hue angle (H_{ab}°) and chromaticity (c^*) were then calculated, according to (Rørå, et al., 1998) as:

$$H_{ab}^{\circ} = \arctan b^* / a^* \quad \text{Equation 4.2}$$

$$C^* = \sqrt{\frac{a^{*2}}{b^{*2}}} \quad \text{Equation 4.3}$$

H_{ab}° ranges from total redness ($H_{ab}^{\circ} = 0$) to total yellowness ($H_{ab}^{\circ} = 90$), whereas the more intense the colour the higher the c^* value (Rørå, et al., 1998).

4.2.4.2 Chemical analyses

Proximate Analysis

The fresh and smoked fish samples were analysed for moisture, protein, fat, salt and ash contents using the methods described in AOAC (2005). Samples were deboned and the heads removed, after which the muscle was minced thoroughly before analysis.

The moisture content of the samples was determined using the air-oven method (AOAC, 32.1.03). Five grams of the fish sample was dehydrated at 105°C for 4 hours to constant weight. The loss in weight during drying was expressed as g water/100 g sample.

The dry ashing method by AOAC (32.1.05) was used to analyse the ash content. About 5 g of the fish sample was weighed into a crucible and incinerated at 550°C for 8 hours in a muffle furnace. Results were expressed as g ash/100 g sample.

Protein was analysed using the Kjeldahl method (AOAC 4.2.09). About 2 g of minced fish was digested by sulphuric acid in the presence of a catalyst. The digest was rendered alkaline, then the liberated ammonia was distilled and titrated with hydrochloric acids. The crude protein was obtained by multiplying the nitrogen content by 6.25. The results were expressed as g protein/100 g sample.

The Soxhlet method (AOAC 4.5.01) was used to evaluate the fat content. Fat was extracted from 5 g of the minced sample with petroleum ether (boiling point of 40-60°C) for 8 hours. The solvent was removed from the extract by evaporation (in a fume chamber) and the residue was weighed and reported as fat. The fat content was expressed as g fat/100 g sample.

Sodium chloride (Salt) content

The salt content was determined by weighing 5 g of sample into an extraction bottle, 200 ml of deionised water was added and shaken using a shaker for 50 minutes. 20 ml of nitric acid was then added to 20 ml of the supernatant and titrated with silver nitrate (AOAC, 2000). Results were presented as g NaCl/100 g sample. The salt content in the water phase (Z-value) for smoked mackerel was calculated as:

$$Z - value = \frac{100 \times \% S}{\% M + \% S} \quad \text{Equation 4.4}$$

where: %S is percent salt content and %M is percent water (moisture) content in final product.

pH

The pH was determined following a modified AOAC (2.8.01). About 5 g sample from the fish was homogenised with distilled water. The pH of the homogenate was measured with a glass electrode pH meter. The pH was expressed in 10 g/100 ml water.

Total Volatile Base

The steam distillation method of Pearson (1970) was used to analyse the total volatile base (TVB) content of the samples. 10 g minced fish sample was added to the distilling flask, into which 2 g magnesium oxide and 300 ml distilled water were added. 25 ml of 2% boric acid and a few drops of screened methyl red indicator were added to a 500 ml receiving flask. The macro-Kjeldahl apparatus containing the mixture was heated for 10 minutes and using the same rate of heating, distillation was carried out for 25 minutes. The distillate was then titrated with 0.1 N sulphuric acid. The titre (less blank) was multiplied by 14 to obtain the TVB content as mg N/100g flesh.

Histamine analysis

The levels of histamine were measured following the method by Hardy & Smith (1979). 10 g of minced flesh (without skin, scales, bones or other undesirable parts) was put into a blender, into which 100 ml of freshly prepared 2.5% trichloroacetic acid (TCA) was added. The mixture was homogenised for 3 minutes. The solution was filtered, and the volume recorded. The TCA sample solution was then neutralised to pH 7 with 1 N KOH and 0.2 N HCl. The new volume was then recorded. A narrow chromatography column was packed with 1 g Amberlite CG-50 resin and washed with 150 ml acetate buffer, then the eluent was drained so that the surface of the liquid aligned the surface of the resin. 75 ml of the neutralised TCA sample solution was then applied to the prepared column and the flow adjusted to approximately 1.5ml/min. The

surface of the Amberlite CG-50 was drained and 100 ml acetate buffer was applied to remove interfering substances. The histamine was eluted with exactly 25 ml of 0.2 N HCl and the eluate was collected in a 50 ml beaker. 1 ml of the HCl eluate was added to 15 ml 5% Na₂CO₃ in a stoppered test tube previously chilled in an ice water bath. 2 ml of chilled diazo reagent was added to the mixture and allowed to stand at 0°C for 15 minutes prior to absorbance measurement. A blank determination was performed using similar volume of 2.5% TCA. The absorbance was measured at 495 nm using distilled water as a reference. A standard curve was prepared by using 1 ml aliquots of a standard histamine solution (0-80 µg histamine/ml 0.2 N HCl). 80 µg/ml (2 mg/25 ml) in the acid eluent. The results were expressed as mg/kg.

4.2.4.3 Microbiological analyses

Fish samples (fresh and smoked) were analysed for total count of aerobic mesophiles (TVC), coliforms (faecal coliforms and *Escherichia coli*), staphylococci, *Clostridium perfringens* and yeasts and moulds as per the Ghana Standards Authority quality criteria (Failler, Yolaine, & Asiedu, 2014).

The rinse method (APHA, 2015) was used in sample preparation. 25 g of each sample (including head, skin, bones and muscle) was aseptically weighed into a sterile stomacher bag, into which 225 ml of 0.1% peptone water was added. The bag was then massaged by hand for about 1 minute. Serial dilutions were performed on the rinse fluids and homogenised tissue samples for the various microbial count tests, per the methods described by the International Commission for Microbiological Specifications for Foods (ICMSF, 1986).

Total mesophilic count (TVC) was determined by plating aliquots on plate count agar (PCA) using pour plate technique. Enumeration of TVC was performed after 24 hours incubation

under aerobic conditions at 37°C. Two replicates of at least two dilutions with 25-250 discrete colonies were enumerated following incubation.

Fecal coliform and *E. coli* were enumerated and detected by spread plating three serial decimal dilutions of the rinse fluid on Levine Eosin Methylene Blue (EMB) agar (Oxoid CM0069) and incubated at 37°C and 44°C for 24 hours respectively. Plates with 20-200 metallic green colonies were counted and sub-cultured on MacConkey agar (Oxoid). Faecal coliforms fermented lactose and appeared as pale pink colour on MacConkey, whereas *E. coli* colonies appeared red. Five *E. coli* colonies were sub-cultured on Sorbitol MacConkey (SMAC) agar (Merck), where they appeared as pinpoint, pale pink colonies. These suspected colonies were then purified on nutrient agar and then transferred unto Triple sugar iron agar (TSI) slants, Simon's citrate slants and Sulphur-Indole-Motility (SIM) agar. *E. coli* colonies were indole positive, gas positive, H₂S negative, citrate negative and could ferment glucose and sucrose.

Staphylococci were determined by spread plating three serial decimal dilutions of the rinse fluid on Baird-Parker agar (Oxoid CM275) supplemented with egg yolk tellurite emulsion (Oxoid). After incubation at 37°C for up to 24 hours, plates containing 20-200 typical staphylococcal colonies (black, circular) were counted. Up to 5 colonies on each plate were sub-cultured on nutrient agar (Oxoid CM003), gram stained and tested for catalase activity. All typical staphylococci were catalase positive and were gram positive stacked cocci. *S. aureus* colonies were circular, convex, grey-black to jet-black with an off-white margin. They were also coagulase positive (APHA, 2015).

The pour plate technique was used to enumerate *C. perfringens*. Serial dilutions were made using 0.1 ml aliquots on TSC (tryptose-sulfite-cycloserine) agar (Oxoid CM0587)

supplemented with egg-yolk and TSC supplement (Oxoid). After the agar had dried slightly, the surface was overlaid with 5 ml of TSC agar and incubated upright in an anaerobic jar containing an aerobic gas generating kit (Oxoid anaerogen) and incubated at 37 °C for 24 hours. Plates containing 20-200 black colonies with opaque halos were selected and counted. To confirm presumptive positive *C. perfringens* colonies, 5 black colonies were selected and tested for motility in SIM agar and for nitrate reduction in nitrate broth (Fluka 72548). *C. perfringens* reduced nitrate and was non motile (APHA, 2015).

Yeast and moulds were analysed from the aliquots by pour plating onto Potato Dextrose Agar (Oxoid, CM0139) and incubated at 25 °C for 3-5 days. Plates containing 20-200 colonies were counted. All microbial counts were expressed as log CFU/g sample.

4.2.4.4 Sensory evaluation

A Quantitative Descriptive Analysis (QDA®) method was carried out to compare the sensory descriptive profiles of smoked mackerel and barracuda from the two different kilns (Lawless & Heymann, 2010). Two different descriptive tests were carried out for the mackerel and barracuda. An eleven-member panel with prior training in Quantitative Descriptive test were recruited for the test. The assessors were trained, in three sessions, to describe, assess and score sensory attributes of smoked fish i.e. the appearance, aroma, flavour, texture (in hand), mouthfeel and aftereffect of samples. The assessors developed two different lists of attributes to describe the smoked mackerel and barracuda (Table 4.1 and 4.2). Food and non-food reference materials suggested by the panel were used to further comprehend the descriptive list of attributes to ensure that all descriptors were used in the same way by all panelists. Further training involved a ranking test, where assessors were given coded products and asked to rank

based on intensities for the generated attributes. This ensured that the panelists understood and used the attributes generated in the same way.

For assessments and evaluations, samples of fish were taken out from each batch of mackerel and barracuda and cut into pieces while frozen to enable easy and uniform cuts and prevent any possible structural disintegration due to flaking. The head and tail fin portions of the samples were cut off and discarded. The body was then cut transversely into two equal parts. Each part was then split along the backbone into two symmetrical halves, de-boned and gutted, and further divided to obtain four equal portions. The prepared samples were transferred into appropriately labelled 80 cc plastic containers with lids and left out at ambient temperature to thaw to about $18\pm 2^{\circ}\text{C}$ before serving to assessors. Samples were presented to assessors, in triplicate, in a randomised balanced order using the William's design in Compusensecloud (Compusensecloud®, Guelph, Ontario, Canada). Samples were served in a monadic sequential order. The assessors used Compusense Cloud® (Compusensecloud ®, Guelph, Ontario, Canada) to score the intensities of the different attributes defined on a 15 cm line scale (Lawless & Heymann, 2010). A total of 26 and 24 sensory descriptors were generated to describe the smoked mackerel and barracuda respectively (Table 4.1 and 4.2).

4.2.5 Statistical analysis

For statistical purposes all microbiological data that were below the limit of detection (LOD) were assumed to be half of the respective LOD. Statistical analysis was performed using Microsoft Excel 2016 and XLSTAT (Addinsoft, New York, USA). Analysis of variance (ANOVA), with stepwise comparison was performed using Tukey's HSD test at the 5% level of significance. Multivariate comparison of different variables and samples was performed

using Principal Component Analysis (PCA) on means to identify differences and similarities between the samples.

Table 4.1: Sensory descriptors developed for smoked mackerel

Sensory descriptor	Term	Definition	Anchor
Appearance (skin of fish)	Glossy	Having a shiny surface	Dull / Shiny
	Oily	Amount of oil on the surface of the sample	Not / Very
	Wrinkle	Depth of folds on the skin	Not / Very
	Iridescent	Typical gold, silver, brown and black appearance of traditionally smoked fish	Not / Very
Appearance (muscle of fish)	Flaky	Sample appears it will crumble when touched	Not / Very
	Cream colour		Not / Very
	Dry	Absence of moisture	Not / Very
	Brown colour		Not / Very
Aroma	Smoked fish	Characteristic aroma of traditionally smoked fish	Not / Very
	Fresh tuna-like	Characteristic aroma of fresh tuna fish	Not / Very
	Dry herring-like	Characteristic aroma of dried herrings	Not / Very
Flavour	Umami	Basic taste	Not / Very
	Smoked fish	Characteristic flavour of smoked fish	Not / Very
	Salty	Basic taste	Not / Very
Texture-in-hand	Tough	Not easily compressed with the hand	Not / Very
	Flaky	Ease with which the fillet crumbles into pieces	Not / Very
	Astringent	Dry feel in the mouth	Not / Very
Mouthfeel	Juicy	How much fluid oozes out during mastication	Not / Very
	Chewy	Needs to be crushed a lot before it is swallowed	Not / Very
	Tough	Not easily compressed in the mouth	Not / Very
	Fibrous	Forms a fibrous mass in the mouth when chewed	Not / Very
	Umami	Basic taste	Not / Very
Aftereffect	Salty	Basic taste	Not / Very
	Salivation	Production of saliva in mouth	Not / Very
	Residue	Presence of fibrous pieces of fish in mouth	Not / Very
	Smoked fish	Lingering smoked fish taste	Not / Very

Table 4.2: Sensory descriptors for smoked barracuda

Sensory descriptor	Term	Definition	Anchor
Appearance (<i>skin of fish</i>)	Soft	Sample gives the impression that it will be easily compressed when touched	Not / Very
	Oily	Presence of oil on the surface of the sample	Not / Very
	Wrinkle	Having folds on the skin	Not / Very
	Iridescent	Typical gold, silver, brown and black appearance of traditionally smoke fish	Not / Very
Appearance (<i>muscle of fish</i>)	Cream colour		Not / Very
	Dry	Absence of moisture	Not / Very
Aroma	Soft	Sample gives the impression that it will be easily compressed when touched	Not / Very
	Smoked barracuda	Characteristic aroma of smoked barracuda fish	Not / Very
	Fried	Characteristic aroma like that of fried fish	Not / Very
	Smoky	Characteristic aroma of burnt palm nut chaff	Not / Very
Flavour	Umami	Basic taste	Not / Very
	Smoked fish	Characteristic flavour of traditionally smoked fish	Not / Very
	Fried	Characteristic aroma like that of fried fish	Not / Very
Texture-in-hand	Salty	Basic taste	Not / Very
	Soft	Easily compressed with the hand	Not / Very
	Flaky	Ease with which the fillet crumbles into pieces	Not / Very
	Juicy	Fluid oozes out during mastication	Not / Very
Mouthfeel	Chewy	Needed to be crushed a lot before it is swallowed	Not / Very
	Soft	Easily compressed in the mouth	Not / Very
	Fibrous	Forms a fibrous mass in the mouth when chewed	Not / Very
	Umami	Basic taste	Not / Very
Aftereffect	Salivation	Production of saliva in mouth	Not / Very
	Residue	Presence of fibrous pieces of fish in mouth	Not / Very
	Smoked fish	Lingering smoked fish taste	Not / Very

4.3 Results

4.3.1 Physicochemical quality of fresh and smoked mackerel and barracuda

Fresh mackerel and barracuda samples of mean total lengths 25.2 ± 3.1 cm and 40.7 ± 4.1 cm and corresponding mean weight 225.46 ± 53.39 g and 474.67 ± 182.05 g respectively, were used in the smoking trials. The mean condition factors (K) were 1.49 ± 0.54 and 0.74 ± 0.33 for mackerel and barracuda respectively.

4.3.2 Chemical composition

The mean moisture, fat, protein, ash, sodium chloride, pH, TVB and histamine contents of the fresh and smoked fish samples are presented in Table 4.3. The moisture, protein, fat and ash contents were 71.97, 24.33, 5.51, 1.41 g/100g and 74.72, 21.76, 2.27 and 1.42 in fresh mackerel and barracuda respectively. After smoking, the moisture content of smoked mackerel significantly decreased ($p < 0.05$) by about 53% and 56% in CSM and GSM respectively. The protein and TVB were significantly higher ($p < 0.05$) in smoked samples (i.e. 111% and 282% in CSM and 97% and 238% in GSM respectively) as compared to FM samples. Fat, ash, salt, pH and histamine contents showed no significant differences ($p > 0.05$) between the fresh and smoked samples. For barracuda, the moisture content was also significantly lower ($p < 0.05$) in smoked samples (34% and 40% in CSB and GSB respectively) than fresh ones. The protein, ash and TVB contents were significantly higher ($p < 0.05$) in the smoked samples (102%, 105% and 163% in CSB and 101%, 159% and 197% in GSB respectively) compared to the fresh ones. The fat and salt contents were significantly higher ($p < 0.05$) in GSB and CSB respectively as compared to the FB. There was no significant difference ($p > 0.05$) between the samples in terms of pH and histamine. There were no significant differences ($p > 0.05$) between the smoked samples from the two kilns, with respect to the chemical composition.

Table 4.3: Chemical quality (Mean \pm SD) characteristics of fresh (F), cabin smoked (CS) and gas smoked (GS) mackerel (M) and barracuda(B) (n = 4)

Parameter	Mackerel			Barracuda		
	FM	CSM	GSM	FB	CSB	GSB
Moisture (g/100g)	71.97 \pm 3.10 ^a	34.18 \pm 9.68 ^b	32.00 \pm 10.13 ^b	74.72 \pm 2.85 ^a	49.15 \pm 4.26 ^b	44.75 \pm 5.30 ^b
Fat (g/100g)	5.51 \pm 5.56 ^a	11.23 \pm 2.99 ^a	16.06 \pm 7.04 ^a	2.27 \pm 3.30 ^a	6.94 \pm 2.02 ^{ab}	10.5 \pm 5.37 ^b
Protein (g/100g)	24.33 \pm 1.37 ^a	51.35 \pm 12.47 ^b	47.95 \pm 4.39 ^b	21.76 \pm 0.82 ^a	43.92 \pm 2.87 ^b	43.75 \pm 3.62 ^b
Ash (g/100g)	1.41 \pm 0.10 ^a	3.67 \pm 0.54 ^a	4.09 \pm 2.32 ^a	1.42 \pm 0.16 ^a	2.91 \pm 0.28 ^b	3.69 \pm 0.99 ^b
Salt (g/100g)	0.30 \pm 0.12 ^a	0.44 \pm 0.18 ^a	0.51 \pm 0.25 ^a	0.18 \pm 0.02 ^a	0.49 \pm 0.15 ^b	0.40 \pm 0.13 ^{ab}
pH	5.98 \pm 0.10 ^a	6.00 \pm 0.08 ^a	6.03 \pm 0.10 ^a	6.60 \pm 0.18 ^a	6.65 \pm 0.13 ^a	6.58 \pm 0.10 ^a
TVB (mg N/100g)	26.32 \pm 2.92 ^a	100.62 \pm 25.50 ^b	89.05 \pm 29.92 ^b	30.70 \pm 6.99 ^a	80.85 \pm 31.50 ^b	91.23 \pm 10.83 ^b
Histamine (mg/kg)	11.83 \pm 7.79 ^a	7.23 \pm 6.63 ^a	10.60 \pm 7.64 ^a	4.31 \pm 5.47 ^a	6.85 \pm 4.72 ^a	5.19 \pm 2.82 ^a

^{a, b} Means of each species with different superscripts within a row are significantly different at $\alpha < 0.05$.

4.3.3 Colour analysis

The skin and muscle colour attributes are presented in Table 4.4 and 4.5 for smoked mackerel and barracuda respectively. The skin redness (a^*), yellowness (b^*) and chromaticity (c^*) were significantly higher ($p < 0.05$) in the CSM compared to GSM (a^* , b^* and c^* of 0.97, 7.56, 7.62 and 0.01, 5.29, 5.29 in CSM and GSM respectively). The hue angle (H^{ab}) of 88.83° in the GSM was significantly higher ($p < 0.05$) than that in the CSM (82.70°). There was no significant difference ($p > 0.05$) between the L^* for CSM and GSM. Muscle colour showed no statistical differences between mackerel from the two kilns. For the barracuda, only the skin a^* was statistically different ($p < 0.05$) between the kilns (i.e. 0.95 and -1.78 in CSB and GSB respectively). Muscle a^* and c^* were significantly higher ($p < 0.05$) in the GSB than in CSB (a^* , c^* of 8.49, 11.94 and 2.62, 4.72 in GSB and CSB respectively).

Table 4.4: Skin colour (Mean ± SD) attributes of cabin smoked (CS) and gas smoked (GS) mackerel (M) and barracuda(B) (n = 5)

Colour attribute	Mackerel		Barracuda	
	CSM	GSM	CSB	GSB
Lightness (L [*])	41.74±2.90 ^a	39.34±1.87 ^a	49.73±0.89 ^a	55.21±5.50 ^a
Redness (a [*])	0.97±0.33 ^a	0.01±0.13 ^b	0.95±0.55 ^a	-1.78±0.78 ^b
Yellowness (b [*])	7.56±2.00 ^a	5.29±0.37 ^b	9.80±1.10 ^a	11.31±2.15 ^a
Hue angle (H ^o _{ab})	82.70±1.54 ^a	88.83±0.47 ^b	84.67±2.82 ^a	81.43±3.00 ^a
Chromaticity (c [*])	7.62±2.02 ^a	5.29±0.37 ^b	9.86±1.13 ^a	11.46±2.23 ^a

Table 4.5: Muscle colour (Mean ± SD) attributes of cabin smoked (CS) and gas smoked (GS) mackerel (M) and barracuda(B) (n = 5)

Colour attribute	Mackerel		Barracuda	
	CSM	GSM	CSB	GSB
Lightness (L [*])	10.87±2.95 ^a	15.08±7.40 ^a	15.52±6.00 ^a	14.64±5.32 ^a
Redness (a [*])	1.69±2.50 ^a	0.83±0.52 ^a	2.62±1.32 ^a	8.49±4.54 ^b
Yellowness (b [*])	1.73±1.46 ^a	3.17±2.28 ^a	3.87±2.37 ^a	8.05±4.02 ^a
Hue angle (H ^o _{ab})	56.09±23.93 ^a	72.28±4.63 ^a	52.98±13.93 ^a	44.64±11.83 ^a
Chromaticity (c [*])	2.57±2.75 ^a	3.31±2.30 ^a	4.72±2.59 ^a	11.94±5.48 ^b

^{a, b} Means of each species with different superscripts within a row are significantly different at $\alpha < 0.05$

4.3.4 Microbiological analyses

Faecal coliform was observed in all fresh fish samples. This decreased in smoked samples with CSB having the least (25%). The percent prevalence of *E. coli* was the same (50%) in all samples, except for CSM, which was higher (75%). *C. perfringens* decreased in the smoked mackerel to 25%, when compared to the fresh ones and were absent in smoked barracuda. Yeast and mould were in all samples of FM, GSM and FB, and decreased to 75% in CSM,

CSB and GSB. *S. aureus* was in 50% of FM and FB, but decreased to 25% in CSM and CSB and were absent in GSM and GSB, compared to the fresh samples (Table 4.6).

Table 4.6: Prevalence of microorganisms in fresh (F), cabin smoked (CS) and gas smoked (GS) mackerel (M) and barracuda(B) (n = 4)

Microorganism	% Prevalence (n/N)					
	Mackerel			Barracuda		
	FM	CSM	GSM	FB	CSB	GSB
Faecal coliform	100	50	75	100	25	50
<i>E. coli</i>	50	75	50	50	50	50
<i>C. perfringens</i>	75	25	25	75	0	0
Yeast and mould	100	75	100	100	75	75
<i>S. aureus</i>	50	25	0	50	25	0

The mean counts of microorganisms in fresh and smoked samples is presented in Table 4.7. FM had the higher total counts of 7.5 log CFU/g compared to that of FB which was 5.8 log CFU/g. The faecal coliform, *E. coli*, *C. perfringens*, yeast and mould and *S. aureus* were 3.1, 2.4, 1.9, 3.9, 1.2 and 4.6, 2.2, 1.7, 4.8 and 1.5 log CFU/g in F and FB respectively. The microbial counts decreased in all smoked samples as compared to the fresh samples. *C. perfringens* was statistically lower in CSM (0.7 log CFU/g) and GSM (0.8 log CFU/g) and below the detection limit in the smoked barracuda samples from both kilns. *S. aureus* was not detected in smoked mackerel and barracuda from the AGFS.

Table 4.7: Microbiological quality (Mean \pm SD) of fresh (F), cabin smoked (CS) and gas smoked (GS) mackerel (M) and barracuda(B) (n = 4)

Microorganism (log CFU/g)	Mackerel			Barracuda		
	FM	CSM	GSM	FB	CSB	GSB
Total mesophilic count	7.5 \pm 1.0 ^a	3.0 \pm 2.3 ^a	4.0 \pm 4.2 ^a	5.8 \pm 2.0 ^a	3.8 \pm 2.8 ^a	4.3 \pm 4.4 ^a
Faecal coliform	3.1 \pm 0.9 ^a	2.2 \pm 1.8 ^a	1.6 \pm 1.3 ^a	4.6 \pm 1.5 ^a	2.4 \pm 3.3 ^a	2.4 \pm 2.7 ^a
<i>E. coli</i>	2.4 \pm 2.0 ^a	1.4 \pm 0.5 ^a	1.6 \pm 1.2 ^a	2.2 \pm 2.0 ^a	1.8 \pm 1.3 ^a	1.4 \pm 1.0 ^a
<i>C. perfringens</i>	1.9 \pm 0.8 ^a	0.7 \pm 0.2 ^b	0.8 \pm 0.2 ^b	1.7 \pm 0.7 ^a	ND	ND
Yeast and mould	3.9 \pm 0.4 ^a	3.3 \pm 2.3 ^a	3.8 \pm 0.6 ^a	4.8 \pm 0.7 ^a	3.0 \pm 2.6 ^a	3.0 \pm 2.6 ^a
<i>S. aureus</i>	1.2 \pm 0.6 ^a	0.8 \pm 0.2 ^a	ND	1.5 \pm 1.1 ^a	1.5 \pm 1.6 ^a	ND

^{a, b} Means of each species with different superscripts within a row are significantly different at $\alpha < 0.05$; ND = not detected

4.3.5 Sensory evaluation

The spider web plots in Figures 4.1 and 4.2 describe the sensory attributes of smoked mackerel and barracuda from the two kilns. Of the 26 sensory descriptors, 13 significantly discriminating between the two samples ($p < 0.05$). GSM scored significantly higher ($p < 0.05$) in terms of glossy, iridescent skin appearance (i.e. 6.1 and 11.4 respectively) compared to CSM that scored 0.2 and 4.0 respectively. CSM on the other hand had significantly dry and brown muscles ($p < 0.05$), scoring 12.6 and 9.0 respectively. CSM scored 13.3 compared to a score of 0.0 for fresh tuna-like aroma whereas GSM had a decidedly dry herring-like aroma (scoring 13.1 to 0.0 scored by CSM). CSM had a significantly higher ($p < 0.05$) umami flavour and flaky texture (scoring 10.2 and 8.4 respectively, compared to 3.9 and 5.3 for GSM). CSM had an astringent, tough and fibrous mouthfeel (scoring 7.4, 6.6 and 8.6, compared to GSM scores of 4.0, 4.2 and 3.5 respectively). Finally, CSM left and umami after taste and residue in the mouth (4.0 and 4.5 for CSM compared to 0.7 and 2.0 for GSM respectively).

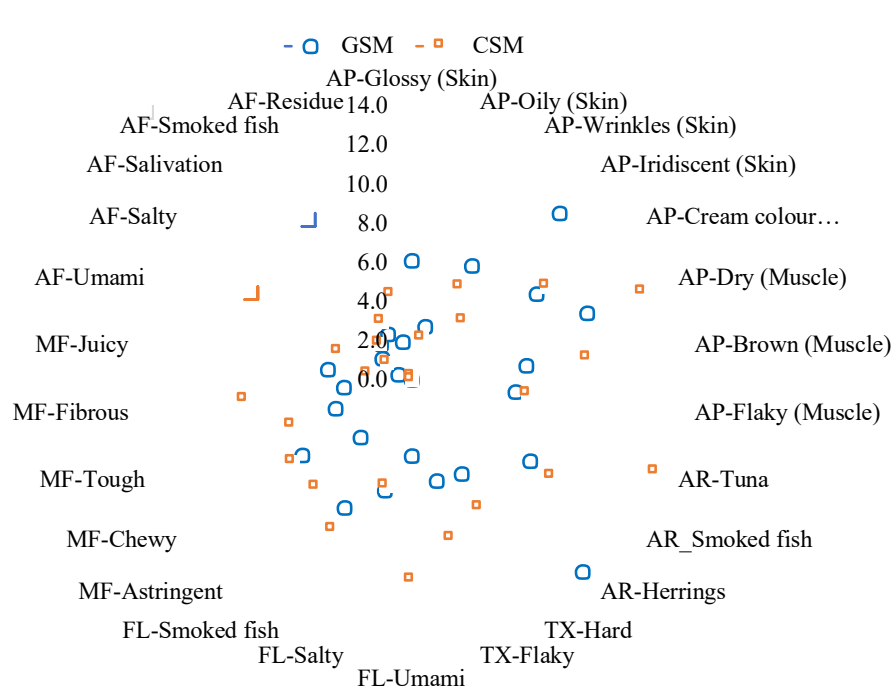


Figure 4.1: Spider web plot of sensory profile of cabin smoked (CS) and gas smoked (GS) mackerel (M) (AP- = Appearance; AR- = Aroma; TX- = Texture; FL- = Flavour; MF- = Mouthfeel; AF- = Aftereffect)

Smoked barracuda similarly had 13 descriptors that were significantly discriminated between the two samples from the two kilns (Figure 4.2). GSB had significantly higher ($p < 0.05$) score for oily skin (3.2) and cream muscle colour (8.4) as compared to CSB (with 0.6 and 6.0 respectively). CSB on the other hand was more wrinkled than GSB (scoring 10.0 and 4.1 respectively). The CSB samples had a generally smoky aroma and flavour (12.4 and 10.3 respectively) whereas GSB had a fried fish aroma and flavour (12.3 and 10.9 respectively). The texture of GSB was flakier (6.3 to 4.4 for CSB). CSB was chewier and more fibrous (9.0 and 7.7 respectively) and left a more smoked fish and residue aftereffect in the mouth (3.3 and 3.4 respectively).

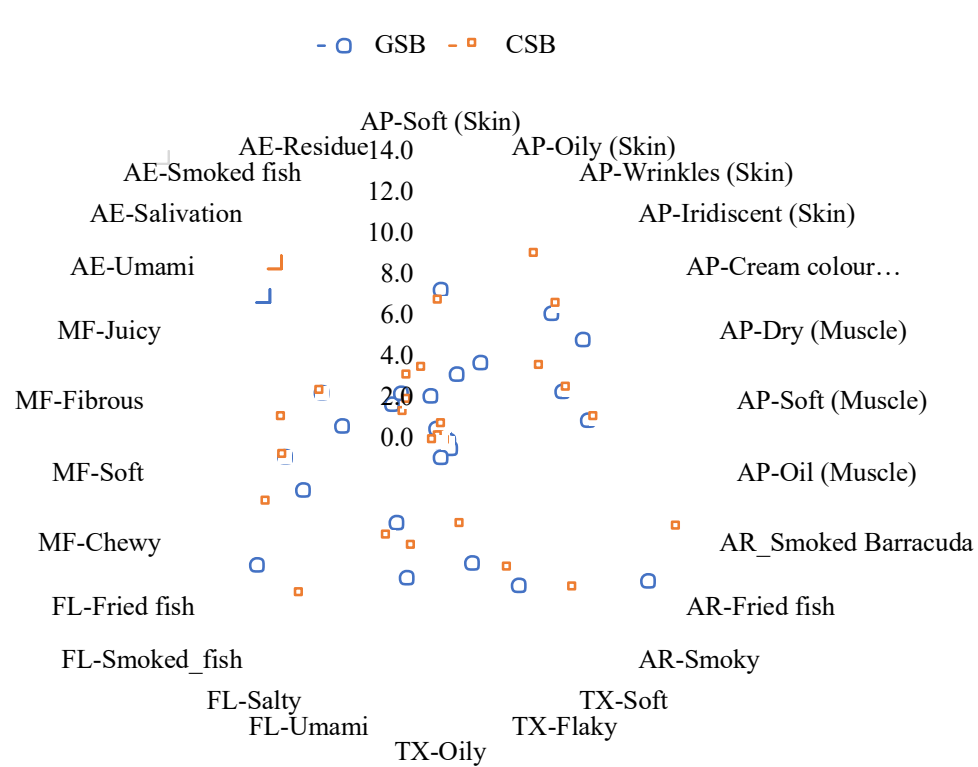


Figure 4.2: Spider web plot of sensory profile of cabin smoked (CS) and gas smoked (GS) barracuda (B) (AP- = Appearance; AR- = Aroma; TX- = Texture; FL- = Flavour; MF- = Mouthfeel; AE- = Aftereffect)

To understand the similarities or differences between sensory attributes of the smoked products from the two kilns, a principal component analysis was carried out. From Figure 4.3, the first principal component (F1) explained 48.77% of the total variation of 66.33% in all the sensory attributes. GSM samples were located to the left and characterised by a glossy appearance, an iridescent colour and a herring aroma. CSM was on the right and described to have brown muscle, a tuna aroma with an intense umami taste. The sample was also flaky, fibrous and left the mouth feeling dry (astringent).

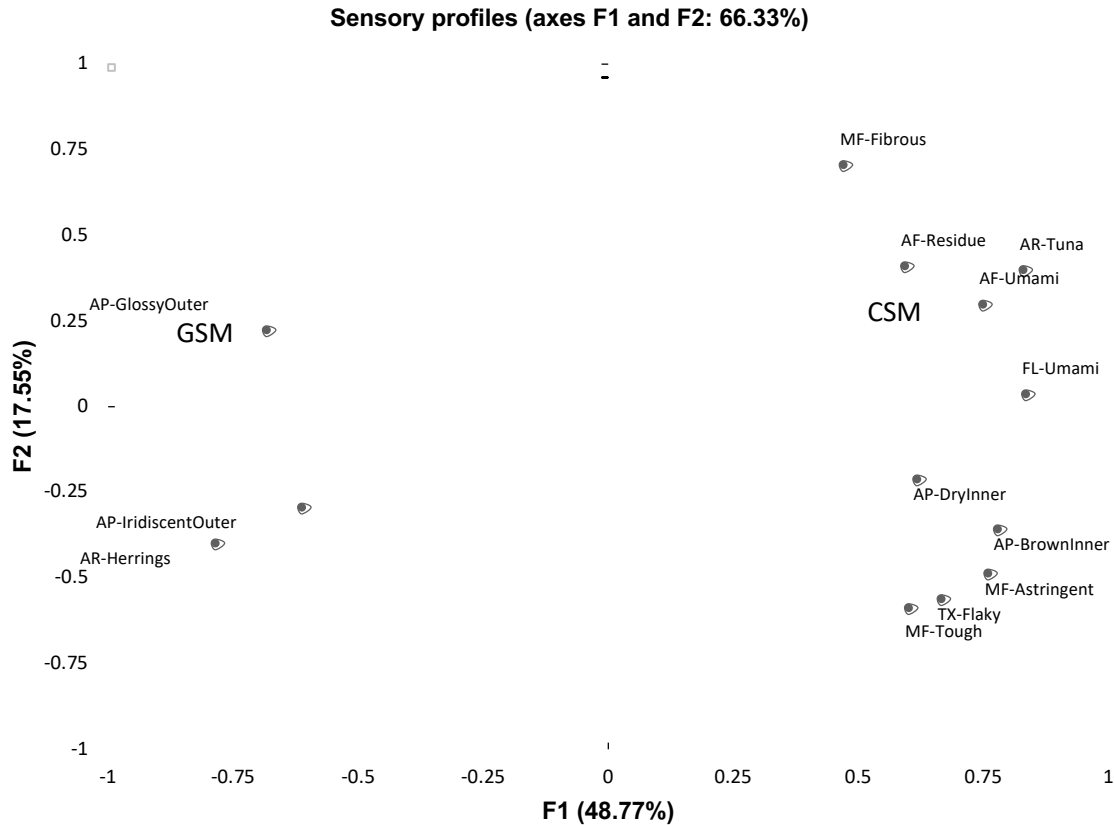


Figure 4.3: Product map showing product and descriptor loading of cabin smoked (CS) and gas smoked (GS) mackerel (M). (AP- = Appearance; AR- = Aroma; TX- = Texture; FL- = Flavour; MF- = Mouthfeel; AF- = Aftereffect)

Based on descriptor and product loading in the product space (Figure 4.4), F1 accounted for 52.95%, out of a total of 68.56%, of the variation in the smoked barracuda samples. These were grouped to the right of the product space. The GSB was best described as having an oily outer appearance, creamy muscle, flaky texture, with a fried fish aroma and flavour. On the other hand, the CSB looked more wrinkled, with an intense smoked barracuda aroma, flavour and left a smoky aftertaste in the mouth. It was also chewier and more fibrous and left fish residues in the mouth.

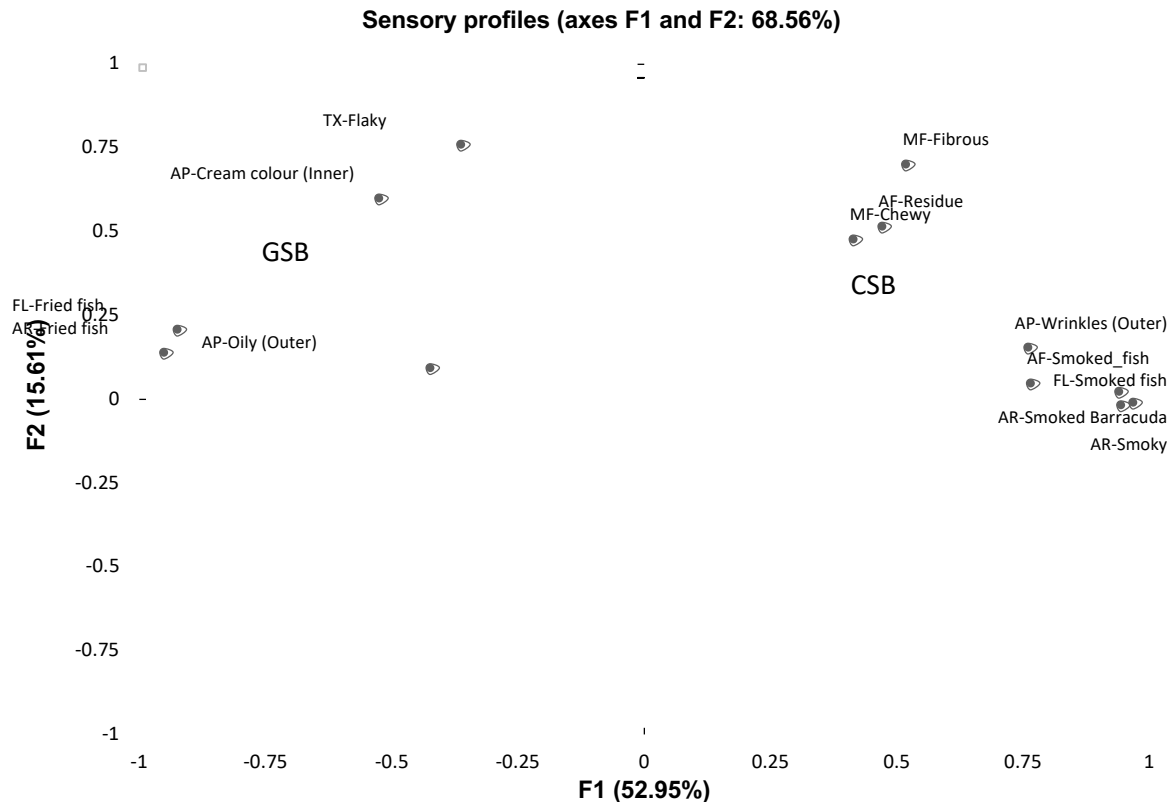


Figure 4.4: Product map showing product and descriptor loading for smoked barracuda. Abbreviations: AP- = Appearance; AR- = Aroma; TX- = Texture; FL- = Flavour; MF- = Mouthfeel; AF- = Aftereffect; GSB for gas smoked barracuda; CSB for cabin smoked barracuda

4.4 Discussion

The nutritional content of fish may differ according to species, body size, season, environmental factors and nutritional status (Al-Reza, et al., 2015). The moisture, fat, protein and ash contents obtained for the fresh mackerel and barracuda in the present study agreed with findings from other studies (Stamatis & Arkoudelos, 2007; Mbarki, Miloud, Selmi, Dhib, & Sadok, 2009; Nogueira, Cordeiro, & Aveiro, 2013; Adeyeye, Oyewole, Obadina, Omemu, & Omoniyi, 2017).

Smoking caused a significant reduction in moisture content (greater than 50% and 30% in smoked mackerel and barracuda respectively), relative to the raw materials. Reports by Goulas

& Kontominas (2005) and Ljubojević, et al. (2016) stated that smoking significantly reduced the moisture content, which supports the findings of this study. A product with a moisture content of less than 65% is considered to meet industrial specification of “smoked finished products” (Cardinal et al., 2001). From the results, both the smoked mackerel and barracuda met this requirement. To inhibit the proliferation of spoilage microorganisms, such as mould, and therefore preserve smoked fish for a longer period, a moisture content of 25% or less (wet weight) has been recommended (Idah & Nwankwo, 2013). This requirement was not met by the smoked products from both kilns and this would suggest that further drying time is needed to make the products more shelf stable.

Smoking resulted in a general increase in the protein, fat and ash contents, which corresponded with an increase in the dry matter content, resulting from dehydration. Idah & Nwankwo, 2013; Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014; Cyprian, Oduor-Odote, & Arason, 2019; Ljubojević, et al., 2016; Adeyeye, Oyewole, Obadina, Omemu, & Omoniyi, 2017 reported similar findings. Protein constituted the greatest percentage of the dry matter in the smoked samples and this agrees with a study by Daramola, Fasakin, & Adeparusi (2007). A study by Suryanti & Suryaningrum (2017) however found significantly lower protein content in smoked tilapia fillets, as compared to fresh ones and attributed this to protein denaturation and subsequent uncoiling of polypeptide chains resulting during smoking. Matsuura, et al. (2015) reported that temperatures of 100°C caused protein denaturation and the loss of essential amino acids. This suggests that the two smoking kilns used possibly operated at a temperature low enough to prevent protein denaturation and keep the quality of the smoked fish. The higher fat content in the smoked fish, as compared to the fresh ones might cause an increase in the energy value of the smoked fish, as suggested by Adeyeye, Oyewole, Obadina, Omemu, & Omoniyi, 2017). Lipid oxidation can however occur, which may affect the flavour, odour and general

quality of the smoked fish (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). The increase in the ash content may be as a result of the deposition of mineral elements present in the salt during brining process (Ikasari, Suryanti, & Suryaningrum, 2017).

The nutritional quality of the smoked products did not differ significantly between the AGFS and the Cabin. This could be as a result of the similar moisture losses observed in smoked fish from both kilns, which allowed the concentration of nutrients not to significantly differ, as observed by Tiwo, Tchoumboungang, Nganou, Pankaj, & Nayak (2019). This was in line with a study by Odoi (2014) who found no significant difference in proximate composition between open fire drum and Cabin kiln smoked mackerel and herring. Chukwu & Shaba (2009), on the other hand, found significant differences in proximate composition of kiln-dried and electric oven-dried catfish in Nigeria. Bouriga, Ben Ismail, Gammoudi, Faure, & Trabelsi (2012) and Sogbesan & Ibrahim (2017) also reported similar findings in nutritional composition of fish smoked using two smoking kilns.

To preserve fish effectively, a suitable salt concentration, in addition to the use of smoke and heat produced during smoking is required. According to Hilderbrand (1992), an appropriate salt concentration of 3.5%, in the water phase, can reduce water activity of smoked products to 0.97 or less that can retard (but not stop) bacteria growth. From the results, all the smoked samples had a salt content less than 3.5% in the water phase. This might imply the products would have a shorter shelf life.

The acidity of fish muscles generally decreases with smoking, as a result of high temperature, absorption of acid from smoke, dehydration and reaction of phenols, polyphenols and carbonyl compounds with protein and protein constituents (Arason, Nguyen, Thorarinsdottir, &

Thorkelsson, 2014). The pH values of smoked samples in this study however increased slightly (but not significantly) from that in the raw samples. Similar findings were made by Bilgīn, Ünlüsayın, Izci, & Günlü (2008) and da Silva, et al. (2008). pH is one of the most important factors that can influence microbial growth and cause spoilage of seafood (Adeyeye, Oyewole, & Obadina, 2016). A pH value of 7 presents optimal condition for microbial growth, as most tolerate pH between 5 to 8 (Nester, Anderson, Roberts, & Nester, 2009). The pH of the smoked mackerel and barracuda in the present study were all within this range and so their shelf life could be affected, thus proper storage is required.

The fitness of fishery products for human consumption is usually determined, chemically, by the levels of total volatile base (TVB). The TVB is the sum of ammonia, dimethylamine, trimethylamine (TMA) and other basic volatile nitrogenous compounds (Koral, Tufan, Başçınar, & Köse, 2015). TVB limit of 25-35 mg N/100 g in the muscle has been specified for unprocessed products from various fish species (EU Directive, 2008). However, according to industry specifications, a TVBN level of < 15 mg N/100g represents fresh fish whereas levels > 35 mg N/100g represents stale fish (Tobin & Gormley, 2016). From the results, both the fresh mackerel and barracuda were below the limits and thus were fit for smoking. The significant increase in the smoked products could be due to the partial dehydration of smoked fish resulting in concentration of the TVBN constituents (Goulas & Kontominas, 2005). The TVB limit in dried fish products is estimated at 100-200 mg N/100g (Özoğul & Özoğul, 2000). The levels of TVB in smoked mackerel and barracuda, in this study, were below this limit. In a related study, Plahar, Nerquaye-Tetteh, & Annan (1999) also found TVB levels of 120.6 and 133.8 mg/100g in freshly smoked Sardinella and anchovy respectively in Ghana. These observations implied that the smoked fish were fit for consumption.

Consumers usually use the colour of dehydrated foods as a quality indicator, that determines their purchase decision (Cyprian, et al., 2015). In fish smoking, the colour of the final product is dependent, to a large extent, on the method and the fuel used (Arason, Nguyen, Thorarinsdottir, & Thorkelsson, 2014). In Ghana, the skin colour of smoked fish ranges from black, dark brown, golden brown or light brown to dirty white, however, consumers prefer golden brown or dark brown colour (Obodai, Muhammad, Obodai, & Opoku, 2009; Asamoah, 2018). From the results, the type of kiln used had a significant impact on the redness, yellowness, hue angle and chromaticity of the smoked mackerel, but only redness in smoked barracuda. This was consistent with results obtained by Asamoah (2018) indicating a significant difference in Cabin and Bradley smoked mackerel. The hue angles for all samples was close to 90°, which correspond to total yellowness, with the cabin smoked mackerel being more intense. This could imply a general acceptability of the products. Yellowness of dried fish muscle gives an indication of lipid oxidation and long storage (Cyprian, et al., 2015). From the results, the muscle colour attributes were not significantly different between the two kilns, except for the redness and chromaticity of the smoked barracuda. Yellowness values were low, and this was in line with the sensory evaluation results.

Fresh or processed seafood are excellent substrates for the growth of most common bacterial agents of food-borne diseases, especially when held at improper temperatures (Asamoah, 2018). In fish, the proposed limit of acceptance for human consumption of total mesophilic counts is 7 log CFU/g (ICMSF, 1986; GSA, 2019). The initial quality of the raw mackerel, in this study, had counts exceeding this limit (7.5 log CFU/g), which might suggest poor handling, whereas that of the barracuda was below the limit (5.8 log CFU/g). The Ghana Standards Authority (GSA) has set limits for *E. coli* and *S. aureus* in fresh and smoked fish at 3 and 4 log CFU/g respectively ; and that for faecal coliform and yeast and mould 2 and 4 log CFU/g

respectively (GSA, 2013; GSA, 2019). The results obtained showed faecal coliform counts above the limits in all samples. The *E. coli* and *S. aureus* counts were below the limits in all samples. Yeast and mould counts were high in the raw materials, with fresh barracuda exceeding the limits (i.e. 4.8 log CFU/g), but smoked samples were within the limits. The results suggest that smoking led to the final products having lower microbial counts than the fresh products. This could have resulted from the combined effects of salting, which lowered the water activity; high temperature drying, which provided a physical surface barrier to the passage of microorganisms, and deposition of antimicrobial compounds, that delayed microbial growth (Arason, Nguyen, Thorarinsdottir & Thorkelsson, 2014).

Two kilns performed similarly, with respect to their smoking capabilities, as the differences were not statistically significant. Smoking also caused a general decrease in prevalence of microorganisms except in the case of *E. coli*, which increased in CSM or remained the same. Faecal coliform and *E. coli* are indicator organisms and their presence could indicate faecal contamination and possible presence of pathogenic bacteria. (Fernandes, 2009; Kombat, Nunoo, Ampofo, & Addo, 2013). The presence of faecal coliforms and yeast and moulds may be a result of their high thermal tolerance, making smoking less effective, or poor adherence to good management practices after smoking (Koral, Tufan, Başçınar, & Köse, 2015). This is consistent with observations by Aheto, et al. (2017) that smoking usually decreases total viable bacteria counts, while other microorganisms like moulds and yeast, may still persist due to their resistance to heat. A study by Marc, et al. (2014) also found high counts of thermo-tolerant coliforms and yeast and moulds in smoked mackerel in Benin. *C. perfringens* have the ability to produce heat-resistant spores that can cause food poisoning (Cortés-Sánchez, 2018). The results from the present study however show counts below the borderline limit of 10^4 (Center for Food Safety, 2014) in both the fresh and smoked samples, an indication of their

good quality. Contrary to this result, Sabry, El-Moein, Hamza, & Kader (2016) found high prevalence of *C. perfringens* in fresh fish, and this posed a public health hazard for both processors and consumers in Egypt.

Good quality fish must have a histamine level below 100 mg/kg (but not exceeding 200 mg/kg in any of the samples) in raw and smoked fish respectively (European Commission, 2005; Codex Alimentarius, 2013), while the US-FDA has set a defect limit of 50 mg/kg (FDA, 2011). Fish samples containing levels of 200-500 mg/100g have been known to produce poisoning when consumed (FDA, 2011; Food Safety Authority of Ireland, 2018). From the results, levels of histamine in both fresh and smoked samples were well below these limits, implying that they were safe for consumption. From the microbial results, *E. coli* and *C. perfringens*, microorganisms implicated in histamine production (FDA, 2011), had low counts and this might be the reason for the low histamine levels recorded. A study by Bomfeh (2016) found histamine levels in smoked mackerel and barracuda ranging from <10-26 mg/kg for freshly smoked fish and 11-450 mg/kg in market samples from Ghana. The levels in the market samples suggested post-processing contamination and probable temperature abuse. Amponsah, et al. (2017) found histamine levels of 63.0 mg/kg in smoked mackerels in Ghana, which was above the US FDA defect limit. Yesudhasan, et al. (2013) also found high concentrations in frozen mackerel and barracuda as compared to fresh fish in Oman and suggested that handling and temperature abuse may have been the cause. Smoking can inactivate the enzyme and microorganisms from continuing to produce histamine but that already formed cannot be eliminated since its heat stable (FDA, 2011) and this might account for the high levels in the study by Amponsah, et al. (2017).

To ensure that a product does not experience market failure, sensory analysis is required. Consumers often demand products that are safe and of good nutritional and sensory quality (Duxbury, 2005). The results indicated that overall, the cabin smoked products had the more traditional smoky appearance, flavour and aroma, as opposed to the gas smoked samples which had a more fried aroma, flavour and appearance. The lower smoky notes in the gas smoked samples may have been as a result of insufficient smoke generation and time. This could affect the acceptability of the product by consumers who are used to the smoked colour, flavour and aroma of traditionally smoked fish in Ghana. The TVBN and sensory scores of the smoked product were in agreement since neither of them indicated spoilage characteristics in the products. The sensory analysis was able to statistically differentiate between the smoked products from the two kilns, which the chemical analysis or colour analysis (of the smoked barracuda) could not achieve.

4.5 Summary of findings

The results of the study indicated smoking improved the physical, chemical, microbiological and sensory quality of smoked mackerel and barracuda. These qualities, with the exception of colour and sensory analysis, could not be statistically differentiated between the products from the two different smoking kilns. The Cabin-smoked products had the more traditional qualities of smoked fish (appearance, odour and flavour) that the gas-smoked products lacked. This was probably due to the indirect smoke generation and/or short contact between the fish and the smoke in the AGFS. Based on these factors, it can be concluded that the Cabin kiln which uses semi-controlled conditions might combine the fuel flexibility of the Chorkor kiln and the efficiency of the gas-fired AGFS kiln.

CHAPTER FIVE

5.0 POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) AND HEAVY METALS IN FRESH AND SMOKED FISH USING THREE DIFFERENT KILNS: LEVELS AND HUMAN HEALTH RISK IMPLICATIONS THROUGH DIETARY EXPOSURE IN GHANA

5.1 Introduction

Smoking preserves fish, or other food products, by exposing it to smoke from smouldering wood or plant materials (FAO/WHO, 2012). In many developing countries, including Ghana, smoking is the most popular way of preserving fish, using traditional fish smoking kilns (Adeyeye & Oyewole, 2016). The most common fuel used by these kilns is firewood, which is burnt at temperatures of 300-700°C (Essumang, Dodoo, & Adjei, 2013).

The smoking process can result in the deposition of beneficial substances like antimicrobial and antioxidant compounds (Arason, Nguyen, Thorarinsdottir & Thorkelsson, 2014). At the same time chemical contaminants like polycyclic aromatic hydrocarbons (PAH), dioxins, formaldehyde, nitrogen, sulphur oxides and heavy metals can be deposited on smoked products (Codex Alimentarius Commission, 2009).

PAHs are a class of persistent organic ecological toxicants which are of health concerns, as they have been observed to be carcinogenic, teratogenic and mutagenic to humans (Codex Alimentarius Commission, 2009; Xia, et al., 2010; Kim, Jahan, Kabir, & Brown, 2013; Essumang, Dodoo, & Adjei, 2012, 2014; Bandowe, et al., 2014; Li, et al., 2016; Ncube, et al.,

2017). PAHs may be present in raw food such as fish, owing to environmental contamination of the water or as a result of lignin pyrolysis in biomass fuels, such as firewood, during fish smoking (Codex Alimentarius Commission, 2009). Other factors that determine the degree of PAH contamination in smoked fish include the type and composition of fuel used (e.g. wood and other plant materials, gas among others), smoking method (direct or indirect), distance between fish and heat source, fat content of fish, duration of smoking and the design of smoking chamber products (Codex Alimentarius Commission, 2009).

The USEPA has identified sixteen priority PAHs and these have been classified as follows: benzo(a)pyrene is a definite carcinogenic (Group 1); dibenzo (a,- h)anthracene is probably carcinogenic (Group 2A); and benzo(k)fluoranthene, benzo(b)fluoranthene, chrysene, naphthalene, indeno(1,2,3-cd)pyrene, and benzo(a)anthracene are possibly carcinogenic (Group 2B). The other PAHs, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, fluoranthene and benzo(g,h,i)perylene, have however not been classified as to their carcinogenicity to humans (Group 3) (IARC, 2018). The EU has set maximum limits (MLs) for benzo(a)pyrene and the sum of four carcinogenic PAHs at 2.0 and 12.0 µg/kg respectively (European Commission, 2011).

Apart from the consumption of smoked fish products, emissions from the combustion of biomass fuels (mainly wood) during the smoking process have been identified as a potential human exposure pathway to carcinogenic PAHs and other substances (Group 2A) (IARC, 2018). Smoked fish processors who usually stay in smoke-filled huts for long periods are particularly at a greater risk of exposure to these carcinogenic emissions, as well as developing other health problems related to the eye, skin and lung (Flintwood–Brace, 2016).

Some potentially toxic heavy metals detected in fresh and smoked fish are Lead (Pb), Cadmium (Cd), Mercury (Hg), Chromium (Cr), Nickel (Ni) and Arsenic (As) (Daniel, Ugwueze, & Igbegu, 2013; Bandowe, et al., 2014). Cd, Pb and Hg have been classified as Group 1, 2B and 3 carcinogens, respectively (IARC, 2018).

In Ghana, traditional fish smoking kilns commonly used include the “Chorkor” kiln (also known as the “Chorkor” smoker), which was introduced in Ghana in 1969 (UNDP/TCDC, 2001) and was an improvement over the existing kilns due to its higher product throughput and fuel efficiency, longer lifespan, shorter operation time and lower labour input. The main drawback of the Chorkor kiln is in relation to the high concentrations of PAHs in the smoked products, resulting from using firewood (Essumang, Dodoo, & Adjei, 2013, 2014; IRI-CSIR, GSA, & Kwarteng, 2016; Bomfeh, et al., 2019). This reduces the quality of fish products and poses health risks to consumers, which often leads to the rejection of smoked products on international markets (Bomfeh, et al., 2019). To address these concerns, the value chain development component of the West Africa Regional Fisheries Programme (WARFP), a World Bank project that ended in 2018, aimed at developing fish smoking technologies that reduce the levels of PAH in smoked fish to conform to international standards and thereby making the product safe for consumption, while at the same time being fuel and cost efficient with minimal adverse health impacts on fish processors. Such technology will increase the marketability of smoked fish products and contribute to the country’s economic growth (The World Bank, 2011).

Currently, two improved kilns, the Ahotor and FAO-Thiaroye Technique (FTT), have been introduced in Ghana (FAO, 2016b; IRI-CSIR, GSA, & Kwarteng, 2016). An assessment of the two kilns showed that the FTT produced good quality fish with PAHs below the EU maximum

limits (MLs), whereas the Ahotor still produced levels higher than the MLs (IRI-CSIR, GSA, & Kwarteng, 2016; Bomfeh, et al., 2019). The improved performance of the FTT could be attributed to the use of charcoal and an external smoke generator (to provide smoke flavoring) as compared to the use of firewood in the Ahotor kiln. The relatively higher cost of construction of the FTT (\approx USD 1,300) (Entee, 2015a), however, might be a deterrent for its adoption in Ghana. The need to explore other kilns is therefore paramount.

Two improved but lesser known kilns, the Abuesi Gas Fish Smoker (AGFS) and the Cabin kiln were therefore explored in this study and compared with the Chorkor kiln. The AGFS is an indirect smoking kiln that relies on liquefied petroleum gas (LPG) for cooking and drying the fish and agricultural wastes like sugarcane bagasse to impart the smoky flavour to the fish (Nunoo, Asiedu, Kombat, & Samey, 2015; Omri, et al., 2019). The Cabin kiln relies on firewood and it is semi-controlled. Both kilns are enclosed units and are expected to ensure minimal exposure of processors to smoke and heat, while offering fish of good nutritional value.

It is therefore anticipated that the adoption of these technologies in Ghana will be of benefit to the fishing industry (for both small scale and industrial processors) and the country as a whole. Prior to their adoption in Ghana, however, it is imperative to assess the quality of smoked products from these technologies in order to protect human health.

This study therefore aimed to: assess the PAH and heavy metal levels in fresh Atlantic chub mackerel (*S. colias*) and European barracuda (*S. sphyraena*) in Ghana's coastal waters; compare the PAH and heavy metal levels in fish smoked using the AGFS and Cabin kilns to

the existing Chorkor kiln using different fuel sources; and determine the possible carcinogenic health risks associated with smoked fish consumption from these kilns.

5.2 Materials and Methods

5.2.1 Fish sampling and preparation

A total of 10 kg each of fresh Atlantic chub mackerel (*S. colias*) and European barracuda (*S. sphyraena*) were purchased, for smoking, from fishers at the Sekondi fish landing site in the Western Region of Ghana in December 2017 and January and July 2018. These species were selected because they are commercially important pelagic fish species that are (1) mainly smoked and consumed locally by a large section of the Ghanaian population, and (2) exported to the EU and the USA (Entee, 2015c).

The fish were then degutted, washed, brined, drained and smoked using the Abuesi Gas Fish Smoker (AGFS), Cabin Smoker (CS) and Chorkor Smoker (ChS) (described in Chapter Three). Two readily available and common hardwood species used for smoking in coastal communities i.e. ‘Afena’ (*Strombosia glaucescens*) and ‘Esa’ (*Celtis mildbraedii*) were chosen as fuel sources in the CS and ChS.

5.2.2 Fish smoking process

The fish was smoked for approximately 4 hours. The smoking was in two phases: cooking and smoking of the fish for about two hours and drying of the samples for another two hours. For the AGFS, pieces of sugarcane were placed in the smoke chamber and lighted during the smoking phase. The AGFS is equipped with a fan that helps with the heat distribution and the drying of the fish. After smoking, the samples were cooled, wrapped in aluminium foil and

transported to the laboratory for heavy metal and PAH analyses. For the moisture and fat content analyses, samples were packed in plastic ziplock bags and transported to the laboratory.

5.2.3 Consumer survey

A survey was undertaken in three communities in the Western region (Abuesi, Sekondi and Aboadze). A simple semi-structured questionnaire was administered to for hundred consumers, both male and female, to obtain information about their fish consumption patterns. There questions were asked:

- Do you consume mackerel/barracuda?
- How much (quantity) do you consume per meal?
- How often do you consume this fish, daily, weekly or monthly?

5.2.4 Analytical methods

5.2.4.1 Moisture and fat content analyses

The moisture and fat contents were analysed following the descriptions in Chapter Four.

5.2.4.2 Heavy metal analysis

The levels of three heavy metals, lead (Pb), mercury (Hg) and cadmium (Cd), in fresh and smoked fish samples (n = 4) were determined for each species. The samples were deboned, de-headed and minced thoroughly (using a blender) before analysis. They were then bagged, labelled and stored in the freezer until analysis. Prior to the analysis, the minced fish samples were thawed and air-dried to constant weight. About 1.0 ± 0.01 g portions of air-dried fish samples were weighed and transferred into 250 ml conical flasks and acid digested with a di-acid mixture of 10 ml concentrated HNO_3 and 10 ml concentrated HCl. This was then refluxed at $95^\circ\text{C} \pm 5^\circ\text{C}$ in a fume chamber until the digestion was completed. Samples were allowed to

cool and diluted with deionised water to the 50 ml mark, then filtered through a Whatman No. 1 filter paper into a 50 ml volumetric flask. About 10 ml aliquots of each digested sample was taken for analysis of Cd, Pb and Hg concentrations by the Anodic Stripping Voltammetry method (ASV) along with standard solutions. Blank samples were also prepared and analysed in the same manner as the samples but without fish samples. The target metals were identified, quantified and their concentrations expressed in parts per billion (ppb). The limits of quantification (LOQ) for Cd, Pb and Hg were 0.001, 0.001 and 0.005 ppb, respectively.

5.2.4.3 PAH analysis

Fish samples were stored at -80°C prior to analyses at the Ghana Standards Authority. Prior to the analysis, the heads and bones of the fish samples were removed. The samples, with their skins on, were then minced thoroughly to achieve sample homogeneity, bagged, labelled and stored at -20°C ready for extraction. The sixteen (16) US-EPA priority PAHs were targeted (Ncube, et al., 2017). All reagents and solvents for the analysis were of HPLC or Ultra-pure grade. This PAH stock solution (10 $\mu\text{g}/\text{ml}$ of 18 polyaromatic hydrocarbons) was diluted in acetonitrile to produce a spiking solution of 1 ppm ($\mu\text{g}/\text{ml}$) (Aheto, et al., 2017). The spiking solution was serially diluted to produce concentrations of 5, 10, 20, 50, 100 and 200 ppb, which were then used to generate a 6-point multi-level calibration curve. The Agilent Bond Elut QuEChERS procedure (Brondi, De MacEdo, Vicente, & Nogueira, 2011) followed by dSPE clean-up technique (Aheto, et al., 2017) were used for PAH extraction. 3.0 g (± 0.05 g) of each minced sample was weighed into a 50 ml centrifuge tube. 12 ml of de-ionised water and 15 ml of acetonitrile were added to the sample, which was then macerated for 2 min. The QuEChERS extraction salt containing 6 g MgSO_4 and 1.5 g NaCl was then added to the samples in centrifuge tubes. The tubes were capped and vortexed for 1 min at 1500 rpm for liquid-liquid partitioning and then centrifuged for 3 min at 3000 rpm. 6 ml of the acetonitrile (ACN) layer

was transferred into a 15 ml centrifuge tube with dSPE clean-up agents containing 150 mg PSA, 300 mg C18 and 900 mg MgSO₄. The mixture was vortexed for 1 min and then centrifuged for 3 min at 3000 rpm. 4 ml of the upper ACN layer was transferred to a 50 ml pear-shaped flask and concentrated to near-dryness using rotary evaporator at below 40 °C. The near-dry extract was then re-dissolved in 1 ml ethyl acetate, and then transferred into a 2 ml autosampler vial, ready for PAH quantification by gas chromatography-mass spectrometry (GC/MS). The limit of quantification (LOQ) for the various PAHs was 0.1 µg/kg (Aheto, et al., 2017).

5.2.5 Human health risk assessment

5.2.5.1 Health risk associated with heavy metals

The health risk associated to heavy metal ingestion was evaluated by comparing the target hazard quotients (THQ) with reference doses given by the US-EPA as:

$$THQ = \frac{EF \times ED \times IFR \times C}{RfDo \times BW \times AT} \times 10^{-3} \quad \text{Equation 5.1}$$

where C is the metal concentration (mg/kg); ED is the exposure duration/life expectancy of 63 years (UNDP, 2018); EF is the exposure frequency of 365 days/year (Bandowe, et al., 2014); BW is the adult body weight of 64.5 kg (WorldData.info, 2019); AT is the average lifespan for carcinogens (365 days/year x exposure duration); RfDo is the oral reference dose (mg/kg/day) and IFR is the fish ingestion rate in Ghana (g/day). RfDo values for lead, mercury and cadmium are 4×10^{-3} , 3×10^{-4} and 1×10^{-3} mg/kg/day respectively (USEPA, 2000). IFR was calculated based on consumer survey as 28.6 g/day, 42.9 g/day and 8.7 g/day for fresh and smoked mackerel and barracuda respectively. The IFR was estimated based on a mean consumption of 100 and 130 g per meal; and frequency of two and three times a week and two times a month for fresh and smoked mackerel and barracuda respectively.

5.2.5.2 Toxicological risk associated with PAH concentrations

The observed concentrations of PAHs in the fish samples were compared to regulatory limits and guidelines to determine their toxicity. Individual PAH concentrations, the sum of all measured PAHs concentrations ($\Sigma 16$ PAHs) and total carcinogenic PAHs i.e. PAH4 (sum of benzo(a)anthracene, chrysene, benzo(a)pyrene and benzo(b)fluoranthene) were assessed. Also, the ratio of the sum of all lower molecular weight (LMW) PAHs (2-3 rings) to that of higher molecular weight (HMW) PAHs (4-6 rings) were used to assess the source of the PAH in the fresh fish samples, whether petrogenic or pyrogenic (Nyarko, Botwe, & Klubi, 2011).

5.2.5.3 Carcinogenic risk (CR) assessment

The CR was calculated using concentrations of the seven carcinogenic PAHs (CPAHs) i.e. benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(b)fluoranthene, indeno(1,2,3-cd)pyrene, dibenzo(a,-h)anthracene and benzo(k)fluoranthene. The dietary daily PAH exposure level (E_D) was assessed for the adult population using Eq. (1) (Bandowe, et al., 2014; Xia, et al., 2010; Li, et al., 2016):

$$E_D = BEC \times IFR_i \quad \text{Equation 5.2}$$

$$BEC = \sum_i^7 C_i \times PEF_i \quad \text{Equation 5.3}$$

where C_i is the concentration of PAHs ($\mu\text{g}/\text{kg}$) in the fish tissue; BEC is the converted sum of seven carcinogenic PAHs based on the potency equivalency factors (PEFs) of BAPeq ($\mu\text{g}/\text{kg}$) (Table 5.1).

The incremental lifetime cancer risk (ILCR) caused by dietary exposure to PAH from daily intake was then calculated using Eq. (3) (Xia, et al., 2010; Li, et al., 2016):

$$ILCR = \frac{E_D \times EF \times ED \times SF \times CF}{BW \times AT} \quad \text{Equation 5.4}$$

where E_D is the daily PAH exposure level ($\mu\text{g}/\text{day}$); SF is the oral cancer slope factor for benzo(a)pyrene [(with geographic mean of $7.3 \text{ mg}/\text{kg}/\text{day}$)⁻¹] and CF is the conversion factor ($10^{-6} \text{ kg}/\text{mg}$) (Li, et al., 2016).

5.2.5.4 Non-carcinogenic risk (non-CR) estimates

The non-CR estimates were based on the Hazard Index (HI) relating to eight non-carcinogenic PAHs (naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene and fluoranthene). The Hazard Index (HI) was calculated using Eq. (4) (Li, et al., 2016).

$$HI = \sum_i^8 \frac{C_i \times IFR \times CF \times ED \times EF}{BW \times AT \times RfDo} \quad \text{Equation 5.5}$$

where $RfDo$ is the oral ingestion reference dose ($\text{mg}/\text{kg}/\text{day}$)⁻¹ (Table 5.1) and C_i is the PAH concentration ($\mu\text{g}/\text{kg}$). Values for estimating CR and HI are presented in Table 5.1.

Table 5.1: Oral ingestion reference dose (RfDo) and potency equivalency factor (PEFs) used in human intake model for estimating cancer risk and hazard index

PAHs	PEF (USEPA, 2013)	RfDo (mg/kg/day) (Li, et al., 2016)
Naphthalene	-	0.04
Acenaphthalene	-	0.06
Acenaphthene	-	0.06
Fluorene	-	0.04
Phenanthrene	-	0.03
Anthracene	-	0.03
Fluoranthene	-	0.04
Pyrene	-	0.03
Benzo(a)anthracene	0.1	-
Chrysene	0.001	-
Benzo(b)fluoranthene	0.1	-
Benzo(k)fluoranthene	0.01	-
Benzo(a)pyrene	1.0	-
Indeno(1,2,3-cd)pyrene	0.1	-
Dibenzo (a,- h)anthracene	1.0	-

5.2.6 Data analysis

Statistical analysis was performed using XLSTAT (Addinsoft, New York, USA) and IBM SPSS version 23. Comparisons of means of moisture and fat contents, across kilns and species were tested by two-way ANOVA followed by Tukey's HSD test at the 5% significance level. All PAH and heavy metal concentrations were expressed as $\mu\text{g}/\text{kg}$ and mg/kg wet weight respectively. For statistical purposes, individual PAH and heavy metal concentrations that were below the limit of detection (LOD) or limit of quantification (LOQ) were assumed to be half of the respective LOD or LOQ. Normality of the PAH data was assessed using the Shapiro-Wilk test. The data were not normally distributed, even after log transformation; therefore, only nonparametric tests were used. The Kruskal-Wallis with Dunn's multiple comparison tests were performed to detect significant differences between raw and smoked samples from the different kilns. Results are presented as mean \pm standard deviation.

5.3 Results

5.3.1 Quality of fresh and smoked fish

Fresh mackerel and barracuda measuring 25.2 cm and 40.7 cm in total length and weighing 225.46 and 474.67 g respectively were smoked using the Cabin (C), Chorkor (Ch) and Gas (G) smoking kilns. The moisture and fat contents of fresh mackerel and barracuda are presented in Tables 5.2 and 5.3. Fresh mackerel had moisture and fat contents of 71.31 and 7.12 g/100g respectively (Table 5.2). The smoked mackerel samples had a moisture content significantly lower ($p < 0.05$) than in the fresh fish. The fat contents were higher in the smoked samples but only the GSM was significantly higher (21.53 g/100g) compared to the fresh samples.

Table 5.2: Chemical quality (Mean \pm SD) characteristics of fresh (F), gas smoked (GS) and Afena (A) and Esa (E) cabin smoked (CS) and Chorkor smoked (ChS) mackerel (M)

Parameter	Fresh (n = 5)		Smoked (n = 6)			
	FM	Chorkor		Cabin		AGFS
		A-ChSM	E-ChSM	A-CSM	E-CSM	GSM
Moisture (g/100g)	71.31 \pm 2.24 ^a	35.45 \pm 5.07 ^b	35.70 \pm 1.71 ^b	27.19 \pm 7.60 ^b	34.18 \pm 8.96 ^b	32.00 \pm 9.38 ^b
Fat (g/100g)	7.12 \pm 3.93 ^a	15.20 \pm 2.39 ^a	10.75 \pm 4.33 ^a	13.95 \pm 2.81 ^a	11.23 \pm 2.78 ^a	21.53 \pm 3.01 ^b

^{a, b} Means of with different superscripts within a row are significantly different at $\alpha < 0.05$.

The fresh barracuda on the other hand had moisture and fat contents of 75.52 and 2.32 g/100g respectively (Table 5.3). The moisture and fat contents in the smoked barracuda behaved similarly like in the smoked mackerel. The GSB had a significantly lower ($p < 0.05$) moisture content than the A-CSB (43.14 and 54.11 g/100g respectively). The fat content was also significantly higher ($p < 0.05$) in the GSB (9.66 g/100g).

Table 5.3: Chemical quality (Mean \pm SD) characteristics of fresh (F), gas smoked (GS) and Afena (A) and Esa (E) cabin smoked (CS) barracuda(B) (n = 5)

Parameter	Fresh		Smoked	
	FB	A-CSB	E-CSB	GSB
Moisture (g/100g)	75.52 \pm 0.94 ^a	54.11 \pm 1.10 ^{bc}	49.32 \pm 3.93 ^{bc}	43.14 \pm 5.23 ^{bd}
Fat (g/100g)	2.32 \pm 1.79 ^a	6.16 \pm 2.87 ^a	6.69 \pm 1.86 ^a	9.66 \pm 2.57 ^b

^{a, b} Means of with different superscripts within a row are significantly different at $\alpha < 0.05$

5.3.2 PAHs and heavy metal levels in fresh fish

The total PAH in FM ranged between 0.87 to 23.72 μ g/kg, with a mean of 11.27 μ g/kg. For individual PAH compounds, the concentrations ranged from below detection limits (chrysene and benzo(b)fluoranthene) to 2.5 μ g/kg (anthracene) in FM. The benzo(a)pyrene in fresh

mackerel ranged from below detection limits to 0.80 ug/kg, with a mean of 0.20 ug/kg. The sum of four carcinogenic PAHs (PAH4) was 0.40 ug/kg.

FB had total PAH concentrations ranging from 0.80 to 30.95 µg/kg, with a mean of 13.56 µg/kg. Chrysene, benzo(a)pyrene and benzo(b)fluoranthene were below detection limits, with phenanthrene having the highest concentration of 3.04 µg/kg. The PAH4 averaged 0.45 ug/kg.

The lower molecular weight (LMW) PAHs accounted for 74 and 83% of the total PAHs in FM and FB respectively. The \sum LMW-PAH/ \sum HMW-PAH ratios were 2.96 and 5.21 for FM and FB respectively.

The concentrations of Cd, Pb and Hg were below their detection limits of 0.001, 0.001 and 0.005 mg/kg, respectively, in both FM and FB.

Table 5.4: Concentrations (ug/kg wet weight) of individual PAHs in fresh (F), gas smoked (GS) and Afena (A) and Esa (E) cabin smoked (CS) and Chorkor smoked (ChS) mackerel (M). (Mean ± SD)

PAH	Fresh (n = 5)		Smoked (n = 6)			
	FM	Chorkor		Cabin		AGFS
		A-ChSM	E-ChSM	A-CSM	E-CSM	GSM
Naphthalene	1.69±2.32	28.95±15.60	26.91±21.22	28.95±22.13	23.26±21.23	15.17±12.59
Acenaphthalene	0.22±0.21	23.27±18.56	17.99±26.53	15.68±23.00	15.33±21.75	0.95±1.17
Acenaphthene	0.14±0.20	1.32±1.22	1.58±1.92	1.37±1.32	5.14±7.09	0.17±0.19
Fluorene	1.81±2.17	34.77±19.18	23.29±25.09	20.99±16.22	23.11±20.98	2.71±3.71
Phenanthrene	1.97±3.14	38.26±17.94	27.75±26.06	45.15±27.07	53.24±54.73	1.22±1.49
Anthracene	2.50±5.34	131.08±120.79	81.99±97.77	61.58±81.32	108.13±98.85	1.09±2.46
Fluoranthene	0.06±0.02	73.76±48.45	49.59±52.10	39.76±42.72	50.87±46.99	3.83±4.74
Pyrene	0.06±0.02	73.80±48.40	49.44±52.20	39.81±42.67	50.87±46.99	3.83±4.74
*Benzo(a)anthracene	0.10±0.11	27.55±20.17	22.68±19.46	18.56±18.83	39.15±13.26	0.81±1.15
*Chrysene	ND	40.30±34.59	25.59±25.39	16.46±22.33	24.01±26.54	1.01±1.26
*Benzo(b)fluoranthene	ND	38.25±48.13	11.33±19.18	11.11±20.61	1.42±2.16	ND
Benzo(k)fluoranthene	0.25±0.36	27.38±19.85	17.17±19.63	10.60±9.51	9.09±10.93	0.53±0.42
*Benzo(a)pyrene	0.20±0.34	15.51±16.63	5.68±7.36	3.59±3.24	1.29±1.67	0.66±0.61
Indeno(1,2,3-cd)pyrene	0.66±1.36	11.82±10.24	4.90±8.47	5.30±6.49	0.32±0.54	2.53±3.89
Dibenzo(a,h)anthracene	0.78±1.63	11.61±10.34	4.79±8.32	4.61±5.63	0.33±0.42	2.17±3.32
Benzo(g,h,i)perylene	0.72±1.50	11.98±9.90	4.78±8.40	4.93±5.97	ND	2.42±3.68
∑PAHs	11.27±10.98	589.59±378.34	375.42±347.09	328.42±231.71	405.58±273.52	39.13±27.74
∑PAH4	0.40±0.45	121.60±98.88	65.27±65.78	49.71±45.56	65.87±35.09	2.52±2.55

* denotes PAHs used in ∑PAH4 calculation; ND denotes not detected (i.e. below the detection limit of 0.1 µg/kg); ∑PAHs denotes total PAH concentration derived from the sum of individual mass concentrations of all 16 PAH congeners measured; ∑PAH4 denotes sum of benzo(a)anthracene, chrysene, benzo(a)pyrene and benzo(b)fluoranthene.

Table 5.5: Concentrations (ug/kg wet weight) of individual PAHs in fresh (F), gas smoked (GS) and Afena (A) and Esa (E) cabin smoked (CS) barracuda (B) (n = 5) (Mean ± SD)

PAH	Fresh		Smoked	
	FB	A-CSB	E-CSB	GSB
Naphthalene	2.67 ± 2.53	12.36±10.54	17.50±10.41	6.61±2.63
Acenaphthalene	0.72 ± 0.70	26.51±32.84	17.51±11.31	0.44±0.41
Acenaphthene	0.92 ± 1.26	11.59±24.37	0.08±0.07	0.17±0.17
Fluorene	1.65 ± 2.91	17.07±29.47	20.16±12.33	2.05±2.08
Phenanthrene	3.04 ± 4.52	14.52±19.17	30.87±22.93	14.96±19.40
Anthracene	2.19 ± 4.54	32.16±53.51	75.30±64.80	9.80±13.69
Fluoranthene	0.11 ± 0.08	34.00±51.14	58.25±40.24	2.29±1.63
Pyrene	0.11 ± 0.08	33.99±51.14	58.25±40.22	2.29±1.63
*Benzo(a)anthracene	0.30 ± 0.56	13.48±11.14	29.11±16.47	0.44±0.55
*Chrysene	ND	13.47±12.25	35.73±22.78	0.47±0.66
*Benzo(b)fluoranthene	ND	4.78±7.01	1.83±3.23	0.35±0.66
Benzo(k)fluoranthene	0.30 ± 0.48	3.68±3.04	8.41±10.63	0.21±0.24
*Benzo(a)pyrene	ND	2.57±3.54	1.32±2.09	0.32±0.28
Indeno(1,2,3-cd)pyrene	0.45 ± 0.76	3.10±5.24	2.54±5.57	3.56±3.86
Dibenzo (a,- h)anthracene	0.53 ± 0.89	4.76±6.46	2.18±4.76	2.02±2.04
Benzo(g,h,i)perylene	0.42 ± 0.83	5.14±6.98	2.34±5.12	2.56±2.49
∑PAHs	13.56 ± 12.58	233.19±290.49	361.39±155.58	48.53±34.86
∑PAH4	0.45 ± 0.56	34.30±30.51	67.99±32.63	1.57±1.44

* denotes PAHs used in ∑PAH4 calculation; ND denotes not detected (i.e. below the detection limit of 0.1 µg/kg); ∑ denotes total PAH concentration derived from the sum of individual mass concentrations of all 16 PAH congeners measured; ∑PAH4 denotes sum of benzo(a)anthracene, chrysene, benzo(a)pyrene and benzo(b)fluoranthene.

5.3.3 PAHs and heavy metal levels in smoked fish samples

Smoking caused an increase in the concentrations of the individual PAHs with the exception of acenaphthene which was significantly lower ($p < 0.05$) in GSM compared to FM (0.17 and

0.23 ug/kg respectively) (Table 5.4). The mean concentrations of individual PAHs in smoked mackerel ranged from below detection limit of 0.1 ug/kg (benzo(g,h,i)perylene and benzo(b)fluoranthene in E-CSM and GSM respectively) to 131.08 ug/kg (i.e. anthracene in A-ChSM). Naphthalene, acenaphthalene, fluorene, fluoranthene, pyrene and chrysene were significantly higher ($p < 0.05$) in A-ChSM compared to FM. The concentrations of fluorene, phenanthrene, anthracene, benzo(a)anthracene were also significantly lower ($p < 0.05$) in FM compared to E-CSM and A-CSM. Fluoranthene and benzo(g,h,i)perylene concentrations were significantly different ($p < 0.05$) between E-ChSM and E-CSM and A-ChSM and E-CSM respectively. GSM samples significantly differed from A-ChSM, E-CSM, A-CSM with respect to fluorene, phenanthrene, anthracene and benzo(b)fluoranthene concentrations. There were no significant differences ($p > 0.05$) in indeno(1,2,3-cd)pyrene, dibenzo (a,- h)anthracene and benzo(k)fluoranthene between all the smoked samples and FM. The concentration of benzo(a)pyrene were 0.66, 1.29, 3.59, 5.68 and 15.51 ug/kg for GSM, E-CSM, A-CSM, E-ChSM and A-ChSM respectively. The Chorkor smoked samples generally had higher benzo(a)pyrene concentrations than the Cabin smoked samples, irrespective of the firewood type used. There were however no significant differences ($p > 0.05$) in PAH concentrations between the samples smoked with the different kiln and also FM.

The mean total PAH concentrations in smoked mackerel ranged from 39.13 ug/kg in GSM to 589.59 ug/kg in A-ChSM (Table 5.4). The total concentrations in FM and GSM were significantly lower ($p < 0.05$) than in E-CSM and A-ChSM. The PAH₄ concentrations also ranged from 2.52 to 121.60 ug/kg in GSM and A-ChSM respectively. A-CSM, E-CSM and E-ChSM recorded PAH₄ concentrations of 49.71, 65.87 and 65.27 ug/kg respectively. PAH₄ was significantly lower ($p < 0.05$) in FM and GSM with respect to E-CSM and A-ChSM. The A-CSM had about 80% less total PAHs compared to the A-ChSM, whereas the E-ChSM had 8%

less than the E-CSM (the main contributor being anthracene in the E-CSM). The GSM had on average, 91 and 89% less PAH than the Chorkor and Cabin smoked mackerel respectively. Also, the GSB had 87% lower PAH than the Cabin smoked barracuda. The LMW PAHs accounted for between 53 to 56% of the total PAHs of A-CSM, GSM and E-CSM and 44 to 48% in A-ChSM and E-ChSM.

The individual PAH concentrations in smoked barracuda ranged from a mean of 0.08 to 75.30 ug/kg for acenaphthene and anthracene respectively. There were no significant differences ($p < 0.05$) in individual PAH concentrations between the smoked barracuda samples from the different kilns and also the FB for naphthalene, acenaphthalene, acenaphthene, benzo(b)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, dibenzo (a,-h)anthracene and benzo(g,h,i)perylene. The concentrations of fluoranthene, phenanthrene, anthracene, fluorene, pyrene and chrysene were significantly higher ($p < 0.05$) in E-CSB compared to FB. Benzo(a)anthracene had a significantly higher ($p < 0.05$) concentration in E-CSB compared to FB and GSB. Benzo(a)pyrene concentrations were 0.32, 1.32 and 2.57 ug/kg in GSB, E-CSB and A-CSB respectively.

The mean total PAH concentrations ranged from 48.53 to 361.39 ug/kg in GSB and E-CSB respectively (Table 5.5). The Cabin smoked samples (A-CSB and E-CSB) recorded total PAH concentrations that were significantly higher ($p < 0.05$) than in the FB. The PAH₄ concentrations were 1.57, 34.30 and 67.99 ug/kg in GSB, A-CSB and E-CSB respectively. Only E-CSB had significantly higher ($p < 0.05$) PAH₄ concentration than FB. The HMW PAHs contributed 51 and 55% to the total PAHs in CSB and 30% to the total PAHs in GSB.

The concentrations of Cd and Hg were below detection limits (< 0.001 and < 0.005 mg/kg respectively) in smoked mackerel and barracuda from the Cabin, Chorkor and Gas fish smoking kilns, irrespective of firewood used. Lead was present in only one sample each of A-CSM and A-CSB and in two samples of GSM, with mean concentrations of 0.16, 0.23 and 0.59 mg/kg respectively, but absent in A-ChSM, E-ChSM and GSB.

5.3.4 Human health risk assessment and dietary exposure of PAHs

5.3.4.1 Carcinogenic health risk assessment

The carcinogenic risks associated with the consumption of fresh and smoked mackerel and barracuda is presented in Table 5.6. From the results, the toxicity equivalencies based on the seven US-EPA priority carcinogenic PAHs, namely benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene and dibenzo (a,- h)anthracene) in fresh mackerel and barracuda were 1.06 and 0.66 ug/kg corresponding to BaPEQ daily dose of 30.39 and 5.75 ug/day. For the smoked mackerel, the equivalencies ranged from 3.17 ug/kg in GSM to 35.19 ug/kg in A-ChSM, with BaPEQ daily doses of 135.86 and 1508.21 ug/day. That for smoked barracuda ranged from 2.78 ug/kg in GSB to 9.52 ug/kg in A-CSB. An adult Ghanaian (with a life expectancy of 63 years and consumption of 28.6, 42.9 and 8.7 g/day for FM, SM and barracuda respectively) would have an estimated cancer risk of 3.44×10^{-6} , 1.54×10^{-5} , 2.82×10^{-5} , 5.73×10^{-5} , 7.06×10^{-5} and 1.71×10^{-4} associated with FM, GSM, E-CSM, A-CSM, E-ChSM and A-ChSM consumption respectively. The estimates for barracuda were 6.50×10^{-7} , 2.72×10^{-6} , 6.83×10^{-6} and 9.34×10^{-6} , corresponding to FB, GSB, E-CSB and A-CSB respectively.

5.3.4.2 Non-carcinogenic risk assessment

The results of non-carcinogenic risks associated with consumption of fresh and smoked mackerel and barracuda are presented in Table 5.7. These estimates were based on the eight non-carcinogenic PAHs (i.e. naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene and fluoranthene). The hazard indices estimated for fresh mackerel and barracuda were 1.12×10^{-4} and 4.38×10^{-5} respectively. That for smoked mackerel were 5.86×10^{-4} , 6.12×10^{-3} , 4.29×10^{-3} , 5.38×10^{-3} and 6.08×10^{-3} for GSM, E-CSM, A-CSM, E-ChSM and A-ChSM respectively. Smoked barracuda recorded estimates of 1.64×10^{-4} , 1.13×10^{-3} and 6.08×10^{-3} for GSB, E-CSB and A-CSB respectively.

5.3.5 Health risk associated with heavy metals

The health risk associated with heavy metal ingestion was evaluated by comparing the target hazard quotients (THQ) with reference doses given by the US-EPA. From the results, lead was present in only A-CSM, A-CSB and GSM and these corresponded to THQs of 2.67×10^{-2} , 7.58×10^{-3} and 9.85×10^{-2} respectively.

Table 5.6: Estimated carcinogenic risks associated with the consumption of fresh (F), gas smoked (GS) and Afena (A) and Esa (E) cabin smoked (CS) and Chorkor smoked (ChS) mackerel (M) and barracuda (B).

Carcinogenic equivalency	Mackerel						Barracuda (n = 5)			
	FM (n = 5)	Chorkor (n = 6)		Cabin (n = 6)		GSM (n = 6)	FB	Cabin		GSB
		A-ChSM	E-ChSM	A-CSM	E-CSM			A-CSB	E-CSB	
Benzo(a)anthracene	0.01	2.75	0.08	1.86	3.92	0.08	0.03	1.35	2.91	0.04
Chrysene	0.00	0.04	0.00	0.02	0.02	0.00	0.00	0.01	0.04	0.00
Benzo(b)fluoranthene	0.01	3.82	0.01	1.11	0.14	0.01	0.01	0.48	0.18	0.03
Benzo(k)fluoranthene	0.00	0.27	0.01	0.11	0.09	0.01	0.00	0.04	0.08	0.00
Benzo(a)pyrene	0.20	15.51	0.66	3.59	1.29	0.66	0.05	2.57	1.32	0.32
Indeno(1,2,3-cd)pyrene	0.07	1.18	0.25	0.53	0.03	0.25	0.05	0.31	0.25	0.36
Dibenzo (a,- h)anthracene	0.78	11.61	2.17	4.61	0.33	2.17	0.53	4.76	2.18	2.02
BEC (ug/kg)	1.06	35.19	3.17	11.81	5.82	3.17	0.66	9.52	6.97	2.78
E_D (μg/day)	30.39	1508.21	623.63	506.32	249.29	135.86	5.75	82.53	60.37	24.07
Carcinogenic risk	3.44E-06	1.71E-04	7.06E-05	5.73E-05	2.82E-05	1.54E-05	6.50E-07	9.34E-06	6.83E-06	2.72E-06

BEC denotes the converted sum of seven carcinogenic PAHs based on the potency equivalency factors (PEFs) of BAPeq (μg/kg); E_D denotes the daily PAH exposure level (μg/day).

Table 5.7: Estimated non-carcinogenic risks associated with the consumption of fresh (F), gas smoked (GS) and Afena (A) and Esa (E) cabin smoked (CS) and Chorkor smoked (ChS) mackerel (M) and barracuda (B).

PAH	Mackerel					Barracuda				
	FM (n = 5)	Chorkor (n = 6)		Cabin (n = 6)		GSM (n = 6)	FB	Cabin		GSB
		A-ChSM	E-ChSM	A-CSM	E-CSM			A-CSB	E-CSB	
Naphthalene	1.93E-05	3.81E-04	2.64E-04	4.42E-04	6.40E-04	3.00E-04	9.23E-06	4.28E-05	6.06E-05	2.29E-05
Acenaphthalene	1.64E-06	1.38E-04	1.13E-04	1.24E-04	1.93E-04	1.29E-05	1.66E-06	6.12E-05	4.04E-05	1.02E-06
Acenaphthene	1.07E-06	1.01E-05	5.88E-05	1.22E-05	2.41E-05	2.17E-06	2.12E-06	2.68E-05	1.85E-07	3.92E-07
Fluorene	2.07E-05	2.95E-04	3.01E-04	4.04E-04	4.36E-04	5.37E-05	5.70E-06	5.91E-05	6.98E-05	7.09E-06
Phenanthrene	3.00E-05	9.86E-04	1.15E-03	6.24E-04	7.92E-04	3.25E-05	1.41E-05	6.71E-05	1.43E-04	6.91E-05
Anthracene	3.81E-05	1.49E-03	2.74E-03	2.53E-03	9.99E-04	1.92E-06	1.01E-05	1.48E-04	3.48E-04	4.53E-05
Fluoranthene	6.85E-07	4.25E-04	6.40E-04	8.33E-04	9.85E-04	7.80E-05	3.81E-07	1.18E-04	2.02E-04	7.92E-06
Pyrene	9.13E-07	5.68E-04	8.53E-04	1.11E-03	1.31E-03	1.04E-04	5.08E-07	1.57E-04	2.69E-04	1.06E-05
Hazard index	1.12E-04	4.29E-03	6.12E-03	6.08E-03	5.38E-03	5.86E-04	4.38E-05	6.80E-04	1.13E-03	1.64E-04

5.4 Discussion

The moisture content of the smoked fish was significantly reduced so as to preserve the products, while at the same time keeping their sensory properties (Cardinal, et al., 2004; Essumang, Dodoo, & Adjei, 2014). The fat contents in the smoked mackerel and barracuda were higher than in the fresh samples, with only GSM and GSB samples being significantly higher than the fresh samples.

The PAH concentrations in the fresh mackerel and barracuda were all low, compared to that in the smoked samples. Essumang, Dodoo, & Adjei (2013) obtained higher concentrations of PAH in fresh mackerel, compared to the present study, with benzo[b]fluoranthene contributing the highest percentage. This indicates that these fish accumulate low levels of PAHs in the marine environment, which is consistent with findings by Stołyhwo & Sikorski (2005). The maximum levels of benzo(a)pyrene and PAH4 concentration in muscle meat of fish other than smoked fish are used as indicators of potential environmental pollution. However, the current EU regulations has established that PAHs are rapidly oxidised and metabolised in fresh fish and hence do not accumulate in the muscles, thus no maximum level is prescribed (European Commission, 2011). The lower molecular weight (LMW) PAHs constituted the highest percentage of the total PAHs implying that the source of PAH was petrogenic ($\sum\text{LMW}/\sum\text{HMW} > 1.0$) for both the FM and FB. This could be due the higher solubility of LMW-PAHs in water, which makes them more bioavailable, and the ease of metabolism and removal of higher molecular weight PAHs (European Commission, 2011; Bandowe, et al., 2014). Again, the site is within the main fishing harbour and its close proximity to vehicular activities, fuel discharge points, among other activities could have caused petrogenic PAH contamination of the fish (Essumang, Dodoo, & Adjei, 2012).

The total PAH content in the smoked mackerel and barracuda, based on fuel type was of the order Gas < Esa < Afena for Gas-Chorkor and Gas < Afena < Esa in Gas-Cabin combinations. The gas smoked fish samples had up to 83% less PAHs than the Chorkor and Cabin smoked fish samples. This implies that in terms of PAH content, the gas kiln had a better product than the other two kilns. The high PAH content in wood smoked fish could be because of high lignin content of both Esa and Afena (25% and 34.5% respectively) (Oteng-Amoako, 2012; Brauns & Brauns, 2013), which causes them to burn hot (averagely between 345.9-465.8°C) (Essumang, Dodoo, & Adjei, 2013). This may have caused increased production and deposition of PAHs in the exposed fish samples than in the gas smoked samples. Again, the high PAH content in smoked mackerel compared to barracuda might be related to the high fat content in the mackerel samples, which has also been observed by several authors (Codex Alimentarius Commission, 2009; Bandowe, et al., 2014; Essumang, Dodoo, & Adjei, 2013, 2014; Aheto, et al., 2017).

With respect to kiln type, the levels were of the order GS < CS < CHS. The Cabin kiln produced mackerel with lower total PAH than the Chorkor (about 80%), when Afena was used. When Esa was however used for the smoking, the Chorkor had a lower total PAH than the Cabin and this was as a result of the higher percentage of lower molecular weight PAHs in the E-CSM compared to the E-ChSM. The high levels of PAH obtained in the Chorkor and Cabin kilns could be due of the of stacking of several trays of fish and covering the topmost tray in the former, and the closed nature of the latter. These could lead to a buildup of smoke around the fish (Bomfeh, et al., 2019). Again, the high levels from both kilns could be as a result of testing for PAHs from a mince of both the skin and muscles in the fish samples tested. This was because both species are usually consumed with skin on. Stołyhwo & Sikorski (2005) in their

study reported that the traditional kilns mostly produce fish that are heavily smoked, with benzo(a)pyrene concentrations up to about 50 ug/kg, especially on their surfaces.

Essumang, Dodoo, & Adjei (2013) found higher levels of total PAHs in Chorkor smoked sardine, cigar minnows, tuna and mackerel using three different fuel sources (acacia, sugarcane bagasse and mangrove). Aheto, et al. (2017) also found higher levels (ranging between 1156.60 to 4443.40 ug/kg) of total PAHs in market samples of sardines, mackerel and anchoives. Their study however did not differentiate between the types of fuel wood and kiln used. Another study by Essumang, Dodoo, & Adjei (2014) using modified traditional kilns with or without activated charcoal filters found an average of more than 40% reduction in the PAH content of fish smoked with filters, which was less than concentrations obtained in the AGFS in the present study.

The maximum limits (MLs) of benzo(a)pyrene and PAH4 in the muscle meat of smoked fish have been set by the EU at 2 and 12 ug/kg (European Commission, 2011). The lowest concentration of benzo(a) pyrene was measured in GSB (0.32 ug/kg), with the highest in A-ChSM (15.51 ug/kg). From the results, benzo(a)pyrene levels in GSM, GSB, E-CSM, E-CSB were all below the EU recommended MLs. The A-CSB, A-CSM, E-ChSM and A-ChSM were 1.3, 1.8, 2.8, times 7.8 times higher than the recommended limits.

The PAH4 concentration was proposed as the most suitable indicator of PAH contamination in foods (European Commission, 2011). The estimated PAH4 ranged between 1.57 to 121.60 ug/kg for GSB and A-ChSM respectively). In PAH4 levels in only the gas smoked mackerel and barracuda were below the EU limits. For Cabin and Chorkor smoked fish, chrysene and

benzo(a)anthracene concentrations contributed the most to the total PAH₄. The Chorkor smoked mackerel 5.4 and 10.1 times higher (for E-ChSM and A-ChSM respectively).

Several studies have compared the PAHs in smoked fish using different technologies in Ghana and beyond. IRI-CSIR, GSA, & Kwarteng (2016) compared the PAH concentrations of smoked fish from two traditional smoking kilns (i.e. the Chorkor, Morrison) to the Ahoror (an improved kiln) in Ghana. The Chorkor, Morrison and Ahoror recorded benzo(a)pyrene and PAH₄ concentrations of 22 and 84 ug/kg; 30 and 110 ug/kg and 5.9 and 53.1 ug/kg respectively. Aidoo (2017) compared the PAH in mackerel (whole and fillet) from two fish smoking kilns (Cabin and Bradley) in Iceland. The PAH and PAH₄ concentrations were 2.8 and 25.85 ug/kg in the smoked fillets and 1.05 and 8.3 ug/kg in the whole smoked mackerel from the Cabin kiln. The Bradley kiln gave concentrations below the EU MLs. These estimates were lower than in the present study and this could be attributed to the type of wood used and the shorter length of time used for smoking.

Essumang, Dodoo, & Adjei (2013) reported benzo(a)pyrene and PAH₄ concentrations of 41.27 and 452.33; 1.26 and 169.4 and 15.22 and 225.75 ug/kg respectively in acacia, sugarcane bagasse and mangrove smoked mackerel from the Chorkor smoker in Ghana. This supported the fact that different firewood sources influenced the level of PAH in smoked fish. The authors also concluded that the elevated levels of benzo(a)pyrene and PAH₄ in smoked mackerel may have been due, in part to its high lipid contents.

Bomfeh, et al. (2019) also compared barracuda smoked with the FAO FTT, Chorkor and metal drum kilns. Soft and dry-smoked barracuda recorded benzo(a)pyrene concentrations of 0.6, 50.3 and 37.4 ug/kg and 1.8, 61.1 and 69.8 ug/kg respectively in FTT, Chorkor and metal drum

smoked fish. These results were however from the use of different sources of fuel. The FTT used charcoal for cooking and sugarcane bagasse for smoke flavouring, while the Chorkor and metal drum used firewood. Also, the FTT represented indirect smoking while the other two were direct smoking methods and this could account for the different levels of PAH in fish products. This was comparable to results from the present study where the gas smoked barracuda had lower PAH concentrations compared to the Cabin smoked ones that used firewood. Another comparison was made between the FTT and the Chorkor smoker using the same types of fuel for smoking sardines. The FTT recorded benzo(a)pyrene and PAH4 concentrations of 0.2 and 1.5; 1.9 and 37.0 and 7.7 and 28.9 ug/kg for charcoal, *Pterocarpus erinaceus* and *Azadirachta indica* respectively. The Chorkor smoked fish recorded benzo(a)pyrene and PAH4 concentrations that were 51 and 26; 32 and 10 and 8 and 7 times higher than the FTT using charcoal, *Pterocarpus erinaceus* and *Azadirachta indica* respectively as fuels. This also indicates that the type of kiln and fuel used can have an impact on the PAH outcomes, which agreed with the findings from the Cabin and Chorkor smoked mackerel using the Afena and Esa wood for smoking in this study.

Another study by Diei-Ouadi (2013) to test different smoking kilns and fuel sources found high levels of benzo(a)pyrene and PAH4 (4.6 and 7.2 ug/kg) in smoked fish when the FTT was used with LPG gas as the fuel source. This was higher than findings from the recent study, implying that the gas smoker used here performed better than the FTT, when LPG gas was used.

An advantage of the AGFS over the Chorkor, Cabin, FTT and Ahotor kilns, among other traditional fish smoking kilns in Ghana is that it does not rely on firewood or charcoal, derived from burning firewood, as source of fuel. This implies deforestation and its attendant problems could be largely eliminated in the fish smoking industry.

The carcinogenic risk associated with the consumption of fresh mackerel and barracuda for an adult Ghanaian with a life expectancy of 63 years were estimated to be between 3.44×10^{-6} and 6.50×10^{-6} . This implied that an estimated 3 in 1,000,000 and 7 in 10,000,000 adults respectively were potentially at risk of suffering from cancer in their lifetime. The gas smoked mackerel and barracuda estimated 2 in 100,000 and 3 in 1,000,000 adults were likely to suffer from cancer. Likewise, Cabin smoked fish had estimates between 3 to 6 in 100,000 whereas Chorkor smoked mackerel fell between 7 per 100,000 and 2 per 10,000 adults (for Esa and Afena respectively in each kiln). The US-EPA has estimated a 1 in 1,000,000 (ILCR of 1×10^{-6}) chance of additional human cancer given a 70-year exposure time (USEPA, 2004). This level is considered acceptable or inconsequential and compares with risks resulting from 'normal' human activities like diagnostic x-rays, fishing, among others (Xia, et al., 2010). A level of risk of 1 in 100,000 (ILCR of 1×10^{-5}) is considered the carcinogenesis threshold, with 1 in 10,000 or greater (ILCR $\geq 1 \times 10^{-4}$) deemed serious and therefore requiring attention (USEPA, 2004; Xia, et al., 2010; Essumang, Dodoo, & Adjei, 2013). From the results, fresh mackerel and all barracuda samples were within the acceptable limits and may therefore pose a very low risk. This could be due to their lower frequency of consumption. All the smoked mackerel samples were above the limits, with the gas smoked being lowest, followed by the Cabin smoked and the Chorkor smoked the highest potential risks. Based on the risks with respect to the fuel type, it could be inferred that LPG was most suitable, followed by Esa, with Afena being very unfavorable, especially when used in the Chorkor kiln.

Bandowe, et al. (2014) estimated risks of magnitude between 7×10^{-7} to 4×10^{-4} in fresh *Cynoglossus senegalensis*, *Pomadasyd peroteti* and *Drepane africana* from different coastal marine areas in Ghana. A report by Essumang, Dodoo, & Adjei (2013) found 5, 15 and 29 out of 100,000 adults were at risk of developing cancer in their lifetime from consuming Chorkor

smoked mackerel using sugarcane bagasse, mangrove and acacia wood in Ghana. Other smoked fish samples (tuna, sardines and cigar minnow) assessed posed low to moderate risks. The authors concluded that the use of sugarcane bagasse was more suitable for fish smoking compared to the use of hardwood.

The non-carcinogenic risks (hazard index, HI) from the results were all less than one. It could therefore be inferred that consumers of the smoked products were unlikely to experience non-carcinogenic effects, as was also reported in a study by Li, et al. (2016).

The concentrations of Cd, Pb and Hg were below detection limits (< 0.001 , < 0.001 and < 0.005 mg/kg respectively) in both FM and FB. This was consistent with a study by Bandowe, et al. (2014), which found low levels of these metals in fresh fish, with the exception of 2 samples. Smoking had no significant impact on the levels of Cd and Hg (remained below detection limits) in mackerel and barracuda from the Cabin, Chorkor and Gas fish smoking kilns. Lead was present in A-CSM, GSM and A-CSB (0.16 ± 0.32 ; 0.59 ± 0.69 and 0.23 ± 0.45 mg/kg respectively) but absent in ChSM and GSB. The EU has set the limits of Cd in muscle meat of mackerel at 0.10 mg/kg and other fish at 0.05 mg/kg; that for Pb and mercury are 0.30 and 0.50 mg/kg respectively (European Commission, 2015). From the results, only GSM had Pb levels exceeding the recommended limits. The levels in the gas smoked mackerel could be linked to the fact that liquified petroleum gas naturally contain Pb. Adeyeye, Oyewole, Obadina, Omemu, & Omoniyi (2017) and Bandowe, et al. (2014) found metal levels below the EU limits in smoked barracuda and fresh fish respectively.

The target hazard quotients (THQs) calculated for each metal and the sum of all metals for all the fresh and smoked samples were however below 1. This implies that possible health risks

of heavy metals via the consumption of fresh and smoked mackerel and barracuda may be negligible, similar to findings by Bandowe, et al. (2014). However, increased consumption of these smoked fish products would likely lead to increased risks (Bandowe, et al. 2014).

5.5 Summary of findings

The smoking process, while aiding in the preservation of fish, also produces some carcinogenic substances such as PAHs and heavy metals in the processed fish. The fresh mackerel and barracuda were of good quality for smoking and posed minimal carcinogenic risks to consumers. With respect to the kilns, AGFS performed best, by producing smoked products with benzo(a)pyrene and PAH4 concentrations below the EU MLs (2 and 12 $\mu\text{g}/\text{kg}$ respectively). The Cabin also produced smoked mackerel with 77% and 59% lower benzo(a)pyrene and PAH4 (only in Esa smoked fish) than the Chorkor. The levels of benzo(a)pyrene and PAH4 were however greater than the EU's MLs in all Chorkor and Cabin smoked samples (except for benzo(a)pyrene in E-CSM and E-CSB). Based on the frequency and quantities of smoked mackerel and barracuda consumed by an average Ghanaian adult (with a life expectancy of 63 years), the potential carcinogenic risks were of least concern in the gas smoked and all barracuda samples, moderate in the Cabin smoked mackerel and high in the Chorkor smoked mackerel. It could therefore be inferred that the presence of PAHs in the smoked fish was due to the type of kiln, smoking method (direct and indirect) and fuel used (LPG and firewood) for the treatment. Again, the magnitude of the carcinogenic risks depended largely on the fish ingestion rate, with higher benzo(a)pyrene and PAH4 levels not always corresponding to increased risks (as shown in the barracuda). Heavy metal (Hg, Pb and Cd) contamination was negligible in fresh and smoked mackerel and barracuda. This indicated that the smoke emitted might not have contained significant amounts of heavy metals and thus contamination was avoided.

CHAPTER SIX

6.0 THE INFLUENCE OF IRRADIATION AND STORAGE TEMPERATURE ON THE QUALITY AND SHELF LIFE OF SMOKED MACKEREL

6.1 Introduction

Smoked fish is a delicacy enjoyed by a majority of the population in most developed and developing nations, including Ghana. It is as such one of the most traded primary fisheries products (FAO, 2018; Bomfeh, et al., 2019). The value chain of smoked fish, from capture to distribution and marketing, however, faces a number of losses (Akande & Diei-Ouadi, 2010). In Ghana, smoked fish processors encountered quality losses between 11-17% immediately after purchase to just before smoking resulting from poor or no icing of fish and lengthy bargaining (Akande & Diei-Ouadi, 2010). During smoking with Chorkor and other traditional kilns, physical losses (about 3-17%) occurred as a result of some fish getting burnt or dropping into the fire. Finally, after smoking and during subsequent packaging, storage and marketing of smoked fish, quality losses estimated at 37.5% occurred via the development of rancidity in the product, microbial contamination and insect infestation (Kleter, 2004; Akande & Diei-Ouadi, 2010). These losses in smoked fish processing have been valued at USD 60 million annually (Akande & Diei-Ouadi, 2010), a significantly high loss in monetary terms for the processors and the country as a whole.

To reduce quality losses encountered after smoking and extend the shelf life of the products, a number of interventions can be employed. In Ghana and most developing nations, smoked fish

is mostly stored at ambient temperatures and sold unpackaged, and this can result in contamination from microorganisms, mainly air-borne moulds and insect attacks (Akande & Ajayi, 2005; Akande & Diei-Ouadi, 2010). For soft smoked mackerel, the shelf life is limited to between 1-3 days when stored unpackaged at ambient temperatures (Kwarteng, Nsiah, Samey, Boateng, & Aziebor, 2016; Asamoah, 2018). The use of effective packaging and the subsequent storage at the right temperature could ensure that a safe and attractive product is delivered to the consumer (Cyprian, et al., 2015).

The use gamma irradiation (low to medium dose i.e. <1-10 kGy) can be employed to both inactivate pathogenic microorganisms (thereby rendering the product safe), and extend the shelf life of a product (Badr, 2012; Arvanitoyannis & Tserkezou, 2014; Ehlermann, 2014). Foods, depending on the type, can be irradiated from below 1 kGy to more than 10 kGy, but the acceptable dose for fish 3 kGy (EU, 2009). With the development of national and international standards following research outcomes, more than 60 countries allow food irradiation of at least one food product (Badr, 2012). Studies have been conducted on the potential of gamma irradiation in improving the safety and quality of vegetables like garden eggs, mushrooms, poultry, fermented maize and cassava products and seafood in Ghana like sardines, anchovies and shrimps (Nketsia-Tabiri, Adu-Gyamfi, Montford, Gbedemah, & Sefaddeh, 2003; Adu-Gyamfi & Appiah, 2012; Adu-Gyamfi, Torgby-Tetteh, & Appiah, 2012; Adu-Gyamfi, Riverson, Afful, & Appiah, 2014; Akuamoa, Odamtten, & Kortei, 2018; Duah, Emi-Reynolds, Kumah, & Larbi, 2018). Ghana has the requisite capacity, therefore, to use this technology to ensure safe and shelf-stable fish products, not only for the domestic market, but also for export.

The objective of the study was to evaluate the influence of gamma irradiation and different storage temperatures on the physicochemical, microbial and sensory quality of smoked Atlantic chub mackerel (*Scomber colias*).

6.2 Materials and methods

Two batches of frozen Atlantic chub mackerel (200 kg), purchased on February 2019, was used for the assessment. The fish was gutted and subjected to brining, by immersion in an 8% brine solution (fish: brine ratio of 1: 2 w/v) for 30 minutes. After brining, the samples were placed on racks to drain for 30 minutes before smoking. The mackerel were smoked whole for approximately 4 hours in the Abuesi gas fish smoker (AGFS) (Chapter Three). After smoking, the samples were cooled at room temperature for about an hour, after which they were packed into sterile ziplock bags and transported to the laboratory for analysis. The samples were divided into 3 lots of approximately 38 kg each. One lot served as the Control (no irradiation) while the other two were irradiated at 1.5 and 3 kGy each. The samples were further divided into two batches each for storage at refrigerated (2-4 °C) and ambient temperatures (26-30°C). The samples for fatty acid and amino acid composition were frozen at -20°C until they were analysed.

6.2.1 Gamma Irradiation

Gamma Irradiation of smoked mackerel was carried out in a category (IV) wet storage cobalt 60 multipurpose gamma irradiator facility (Type CoS43HH) at the Radiation Technology Centre of Ghana Atomic Energy Commission, Accra, Ghana. The smoked mackerel samples were packed in zip lock bags and placed in cardboard boxes for irradiation. Each box with smoked fish weighed 5 kg. Two doses, 1.5 and 3 kGy were targeted and for each dose, about 44 kg of smoked fish was used. The irradiation was performed at a dose rate of 0.495 kGy/hr

and cartons were turned 180° halfway into the processing time to ensure homogenous distribution of the dose delivered under the same conditions. Doses delivered were confirmed using Ethanol-chlorobenzene dosimeters placed inside and outside the cartons. The delivered doses were 1.65 ± 0.11 and 3.35 ± 0.13 kGy.

6.2.2 Analytical methods

Analyses were conducted on fresh/raw, smoked control and irradiated mackerel samples, in duplicate. Laboratory analyses were conducted at the Food Microbiology and Sensory Laboratories of the Department of Nutrition and Food Science, University of Ghana, FRI-CSIR and the National Food Institute of the Danish Technical University. For shelf life studies, sampling was undertaken on Days 1, 5, 10, 15, 25, 45 and 65.

6.2.2.1 Colour analysis

The skin and muscle colour of smoked mackerel (irradiated and non-irradiated) were measured with a Minolta CR-310 chromameter (Minolta Camera Co., Ltd; Osaka, Japan) (Chapter Four). The colour intensity was described in L^* , a^* , and b^* notation on the CIE LAB colour scale, according to (CIE, 1979).

6.2.2.2 Chemical analyses

The proximate composition (moisture, protein, fat and ash), total volatile base (TVB) and pH of the fresh, smoked and irradiated mackerel were determined using the methods described in Chapter Four.

Peroxide Value (PV)

The peroxide value was analysed using the AOAC method 965.33 (AOAC, 2005). Extracted oil sample (5 g) was dissolved in 30 ml of acetic acid-chloroform (2:1 v/v). Saturated potassium iodide (0.5 ml) was added and the sample was kept in the dark for 1 min, after which 30 ml of water was added. The mixture was slowly titrated with 0.1 M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), while vigorously shaking until the yellow colour disappeared. After this 0.5 ml of 1% starch solution was added, and the resulting mixture was continuously titrated with $\text{Na}_2\text{S}_2\text{O}_3$ until colourless. A blank (T) titration was run alongside the samples. The peroxide value (expressed in milliequivalent peroxide/kg fat, mEq O_2 /kg) was calculated from:

$$PV = \frac{S \times M \times 1000}{W} \quad \text{Equation 6.1}$$

Where: S is ml of $\text{Na}_2\text{S}_2\text{O}_3$ (blank corrected); M is the molarity of $\text{Na}_2\text{S}_2\text{O}_3$ solution and W is the weight of the test sample.

Free fatty acid (FFA)

The free fatty acids content was determined, according to (ISO 660:2000) method, for the fat extracted by the AOAC 4.5.01 method (described in Chapter Four). Sample (2 g) was weighed and dissolved in 40 ml of 95% neutralized ethanol. The mixture was heated on a hot plate till it boiled, and titrated with 0.1 N sodium hydroxide (NaOH). The FFA was calculated as a percentage mass fraction of the oleic acid as:

$$FFA = \frac{V \times c \times M \times 100}{1000 \times m} \quad \text{Equation 6.2}$$

Where: V is the volume (ml) of the standard volumetric NaOH solution used; c is the concentration (mol/l) of the standard volumetric sodium or potassium hydroxide solution used; M is the molar mass of oleic acid (282 g/mol) and m is the mass (g) of the test portion. The free fatty acid (FFA) was expressed as % oleic acid.

Fatty acid composition

The fatty acid composition of the lipid extracts (Bligh & Dyer, 1959) was analysed by gas chromatography according to AOCS (1998). Toluene (100 μL), 200 μL heptane with 0.01% (v/v) butylated hydroxytoluene (BHT) and 100 μL internal standard (C23:0) (2% w/v) were added to about 30 mg of lipid extract. One millilitre of borontrifluoride (BF_3) in methanol was added to the lipid extract mixture and the lipids were methylated in a one-step procedure using a microwave oven (Multiwave 3000 SOLV, Anton Paar, Graz, Austria) with a 64MG5 rotor. The settings for the microwave were 5 min at 500 Watt followed by 10 min cooling. The fatty acid methyl esters (FAMES) were washed with 1 mL saturated NaCl and 0.7 mL heptane with 0.01% (v/v) BHT. The heptane phase was analysed by gas chromatography (Agilent Technology Model 7890A series GC, China) fitted with automatic sampler (Model 7693, Agilent Technology), fused silica capillary column (HP-88, 100 m x 0.25 mm x 0.20 μm film thickness; Agilent Technology), split injector, and flame ionization detector (FID). The carrier gas was helium with a flow rate of 0.38 mL/min and an inlet pressure of 51 psi. The oven temperature program for separation was from 160 to 200°C, 200 to 220°C, and 220 to 240°C at 10.6°C /min. For separation, DB127-7012 column (10 m x ID 0.1 mm x 0.1 μm film thickness, Agilent Technologies, Palo Alto, CA, USA) was used. Injection volume was 0.2 μL in split mode (1:50). All analyses were carried out in duplicate. The result of each fatty acid was expressed as g fatty acid/100 g lipid.

The lipid quality indices, polyene index (PI), atherogenic index (AI) and thrombogenic index (TI) were calculated using the following equations (Rosa & Nunes, 2003; Chaula, et al., 2019):

$$I = \frac{C20:5+C22:6}{C16:0} \quad \text{Equation 6.3}$$

$$AI = \frac{C12:0+(4 \times C14:0)+C16:0}{\sum MUFA + \sum PUFA_{\omega-6} + \sum PUFA_{\omega-3}} \quad \text{Equation 6.4}$$

$$TI = \frac{C14:0+C16:0+C18:0}{(0.5 \times \sum MUFA) + (0.5 \sum PUFA_{\omega-6}) + (3 \times \sum PUFA_{\omega-3}) + (\omega-3/\omega-6)} \quad \text{Equation 6.5}$$

where: MUFA is monounsaturated fatty acids and PUFA is polyunsaturated fatty acids.

Amino acid composition

The amino acid composition was analysed using a Phenomenex EZ:faast amino acid analysis kit (California, USA). About 30 mg of the sample was hydrolysed in 6 M HCl in a microwave oven (Microwave 3000 SOLV, Anton Paar, Austria) for 60 min at 110°C. The hydrolysate was filtered into 1.5 mL screw-cap vial through a cellulose acetate 0.22 µm Q-Max RR syringe filter using a 1 mL syringe. The amino acid composition was determined by liquid chromatography with a mass spectrometry detector (Agilent 1100 series, LC/MSD Trap, Agilent Technologies, Denmark) using a Phenomenex Z:faast 4u AAA-MS column (250 × 3.0 mm, California, USA). The total protein concentration in the samples was calculated by summarizing all the amino acids and subtracting the water incorporated during hydrolysis (18 g H₂O mol⁻¹ amino acid) (Mols-Mortensen, Ortind, Jacobsen, & Holdt, 2017).

6.2.2.3 Microbiological analyses

Fish samples (fresh and smoked) were analysed for the total count of aerobic mesophiles (TVC), coliforms (faecal coliforms and *E. coli*), *Staphylococci aureus*, *Clostridium perfringens* and yeasts and moulds based on the methods described in Chapter Four.

6.2.2.4 Sensory evaluation

The difference-from-control test (Lawless & Heymann, 2010) was used to determine if there were any differences between non-irradiated (control) and irradiated smoked mackerel immediately after smoking and irradiation. 15 panelists were screened to be able to detect and

discriminate between basic tastes. Assessors first tasted a labelled control sample (R) and then proceeded to taste three test samples; Blind control (non-irradiated), Irradiated I (1.5 kGy) and Irradiated II (3 kGy) in a pre-determined randomized order. For each test sample, assessors determined overall how different that test sample was from the labelled control sample on a 6-point labelled category scale with the following categories: 0 = No difference; 1= Very slight difference; 2 = Slight difference; 3 = Moderate; 4 = Very different and 5 = Extreme difference. Overall product differences were evaluated and not attribute differences. Assessments were done in duplicates to give 30 responses for each sample. Check-all-that-apply (CATA) list was provided for panelists to indicate where differences (if any) could be perceived.

6.2.2.5 *Insect infestation*

The samples were visually inspected for insects and pest attacks during storage. This was done prior to samples been sent to the laboratory for microbial and chemical quality analyses.

6.2.3 Data analysis

Statistical analysis was performed using Microsoft Excel 2016 and XLSTAT (Addinsoft, New York, USA). For statistical purposes all microbiological data that were below the limit of detection (LOD) were assumed to be half of the respective LOD. Analysis of variance (ANOVA) was performed, with stepwise comparison, using Fisher's least significant difference (LSD) test at the 5% significance level. Results are presented as mean \pm standard deviation (n = 2).

6.3 Results

Results from the physicochemical, microbiological and sensory analyses are presented below.

6.3.1 Chemical composition

6.3.1.1 Proximate composition

The mean moisture, protein, fat and ash content of the fresh and smoked mackerel are presented in Table 6.1. FM had the highest moisture (64.93 g/100g) and lowest protein (25.30 g/100g), fat (10.15 g/100g) and ash (1.45 g/100g) contents relative to the smoked samples. Freshly smoked and irradiated products (Day 1) were statistically different ($p < 0.05$) from the FM in terms of the moisture and protein contents. Comparing the proximate composition between Day 1 and Day 65 (refrigerated storage) showed a general decrease moisture content by Day 65, with the lowest percentage reduction (16%) in the SM and SMg-3.0 kGy samples (39.33 and 36.30 g/100g respectively by Day 65). The ash, fat and protein contents in SM increased by 22%, 45% and 12% respectively by Day 65. The SMg-1.5 kGy samples remained relatively unchanged during the 65 days, except for the fat content that increased from 11.57 g/100g to 20.46 g/100g (77%). The ash and fat content of SMg-3.0 kGy increased from 1.97 and 11.55 g/100g to 2.49 and 20.19 g/100g (27% and 75%) respectively, whereas the protein content decreased from 45.91 g/100g to 37.56 g/100g by Day 65. The changes between Day 1 and Day 65 were however not statistically different ($p > 0.05$) between the SM and SMg products.

Table 6.1: Proximate composition of fresh (F), smoked (S) and gamma irradiated (g) mackerel (M) for Day 1 and Day 65 of refrigerated storage

Parameter (g/100g)	FM	SM		SMg-1.5 kGy		SMg - 3.0 kGy	
		Day 1	Day 65	Day 1	Day 65	Day 1	Day 65
Moisture	64.93±4.91	46.65±5.80	39.33±9.49	45.98±1.17	45.75±7.29	43.13±2.30	36.30±1.71
Protein	25.30±0.11	37.21±4.10	45.91±3.13	37.56±7.37	38.07±5.19	43.29±0.94	39.39±1.51
Fat	10.15±5.89	12.03±9.01	17.50±2.81	11.57±2.91	20.46±0.58	11.54±2.49	20.19±2.42
Ash	1.45±0.13	1.98±0.11	2.42±0.52	2.15±0.46	2.34±0.46	1.96±0.32	2.49±0.11

6.3.1.2 *Fatty acid composition*

A total of 33 fatty acids were identified and quantified in the fresh and smoked mackerel samples (Table 6.2), with the unsaturated fatty acids being relatively dominant (27), compared to the saturated ones (6). Of the unsaturated fatty acids identified, 18 were polyunsaturated fatty acids (PUFAs), with nine being monounsaturated fatty acids (MUFAs). Among the PUFAs, there were 9 omega-3 fatty acids (ω -3) and 5 omega-6 (ω -6) fatty acids.

The total saturated fatty acids (SFAs) of the irradiated and non-irradiated smoked samples were significantly higher ($p < 0.05$) than in the FM (i.e. 23.01, 35.39, 34.13 and 35.65 g fatty acid/100 g oil sample in FM, SM, SMg-1.5 kGy and SMg-3.0 kGy respectively). Irradiation however did not cause a significant increase ($p > 0.05$) in SFAs, compared to SM. Palmitic, myristic and stearic acid constituted 13.53 to 22.61, 5.07 to 5.70 and 2.79 to 6.23 g fatty acid/100 g of the total lipid content, respectively. Stearic acid was significantly higher ($p < 0.05$) in smoked samples. Arachidic acid was statistically different ($p < 0.05$) in all the fresh and smoked samples, but not detected in SMg-3.0 kGy. The total MUFA was significantly reduced ($p < 0.05$) in the smoked samples (ranging from 20.68 g fatty acid/100 g in SM to 45.29 g fatty acid/100 g in FM). Oleic acid accounted for a higher percentage of the MUFAs, ranging from 9.15 to 15.06 g fatty acid/100 g in SM and FM respectively.

The PUFAs also significantly increased ($p < 0.05$) in the smoked samples, relative to the FM (26.22, 34.59, 35.04 and 36.08 g fatty acid/100 g for FM, SMg-3.0 kGy, SMg-1.5 kGy and SM respectively). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) accounted for the highest percentages (ranging from 6.91 to 7.36 g fatty acid/100 g and 8.78 to 17.02 g fatty acid/100 g respectively) of the ω -3 PUFAs, in both the fresh and smoked mackerels. There were no significant differences ($p > 0.05$) in the EPA for all samples, but DHA was

significantly higher ($p < 0.05$) in the smoked samples, compared to the FM. Again, irradiation at 1.5 and 3.0 kGy had no significant effect on the ω -3 fatty acids, compared to the non-irradiated samples. The total ω -6 PUFAs ranged from 3.54 g fatty acid/100 g in FM to 4.78 g fatty acid/100 g in SMg-1.5 kGy. Linoleic and γ -linolenic acids were only significantly lower ($p < 0.05$) in SM and SMg-3.0 kGy respectively, compared to the FM. Dihomo- γ -linolenic was not detected in FM. The total ω -3 and ω -6 PUFAs was also significantly higher ($p < 0.05$) in the smoked than fresh mackerel.

The ratios of ω -3: ω -6 PUFAs were 5.07, 7.72, 5.55 and 5.49 in FM, SM, SMg-1.5 kGy and SMg-3.0 kGy respectively. The PUFA to SFA ratios were also 1.14, 1.02, 1.03 and 0.97 in FM, SM, SMg-1.5 kGy and SMg-3.0 kGy respectively. The polyene index (PI) ranged from and 1.04 to 1.17 in SMg-3.0 kGy and SMg-1.5 kGy respectively. There were no significant differences ($p > 0.05$) in these ratios for all samples. Atherogenic index (AI) was significantly lower ($p < 0.05$) in FM (0.54) compared to SM, SMg-1.5 kGy and SMg-3.0 kGy (0.79, 0.76 and 0.82 respectively). The thrombogenic index (TI) also ranged between 0.26 to 0.35 in FM and SMg-3.0 kGy respectively, and these were not statistically different ($p > 0.05$) from the other samples.

Table 6.2: Fatty acid composition of fresh (F), smoked (S) and gamma irradiated (g) mackerel (M) (g fatty acid/100 g oil sample)

Fatty acid		FM	SM	SMg-1.5 kGy	SMg-3.0 kGy
Myristic	14:0	5.70 ± 0.07 ^a	5.09 ± 0.40 ^b	5.07 ± 0.12 ^b	5.12 ± 0.04 ^a
Pentadecanoic	15:0	0.40 ± 0.02 ^a	1.48 ± 0.16 ^b	1.28 ± 0.11 ^b	1.41 ± 0.05 ^b
Palmitic	16:0	13.53 ± 0.18 ^a	21.68 ± 1.70 ^b	21.06 ± 0.44 ^b	22.61 ± 0.22 ^b
Margaric	17:0	0.43 ± 0.05	0.47 ± 0.38	0.65 ± 0.07	0.63 ± 0.03
Stearic	18:0	2.79 ± 0.05 ^a	6.23 ± 0.24 ^{bc}	5.56 ± 0.05 ^{bd}	5.89 ± 0.20 ^b
Arachidic	20:0	0.16 ± 0.03 ^a	0.44 ± 0.03 ^b	0.51 ± 0.01 ^c	ND ^d
Σ SFA		23.01 ± 0.19^a	35.39 ± 2.06^b	34.13 ± 0.57^b	35.65 ± 0.07^b
Myristovaccenic	14:1	0.21 ± 0.02	0.21 ± 0.04	0.26 ± 0.01	0.28 ± 0.04
Palmitoleic	16:1 (n-7)	4.10 ± 0.08 ^a	5.89 ± 0.49 ^b	6.08 ± 0.08 ^b	6.07 ± 0.24 ^b
Oleic	18:1 (n-9)	15.06 ± 2.23 ^a	9.15 ± 0.74 ^b	9.89 ± 0.17 ^b	10.21 ± 0.39 ^b
Vaccenic	18:1 (n-7)	3.02 ± 0.11	2.68 ± 0.52	2.73 ± 0.00	2.74 ± 0.03
Gondoic	20:1 (n-9,11)	9.43 ± 1.96 ^a	1.15 ± 0.06 ^b	1.43 ± 0.75 ^b	0.99 ± 0.02 ^b
Paullinic	20:1 (n-7)	0.31 ± 0.02	0.38 ± 0.03	0.38 ± 0.01	0.35 ± 0.00
Setoleic	22:1 (n-11)	12.35 ± 0.08 ^a	0.37 ± 0.10 ^b	1.04 ± 1.01 ^b	0.38 ± 0.02 ^b
Erucic	22:1 (n-9)	ND ^a	0.23 ± 0.11 ^a	0.35 ± 0.14 ^b	0.17 ± 0.07 ^a
Nervonic	24:1 (n-9)	0.81 ± 0.14	0.62 ± 0.07	0.72 ± 0.10	0.75 ± 0.04
Σ MUFAs		45.29 ± 0.92^a	20.68 ± 1.93^b	22.88 ± 1.90^b	21.95 ± 0.15^b
9,12-Hexadecadienoic	16:2 (n-4)	0.37 ± 0.01 ^a	0.75 ± 0.18 ^b	0.62 ± 0.10 ^a	0.75 ± 0.10 ^b
6,9,12-Hexadecatrienoic	16:3 (n-4)	0.40 ± 0.02 ^a	1.59 ± 0.00 ^b	1.47 ± 0.13 ^b	1.57 ± 0.10 ^b
11,14-Octadecadienoic	18:2(n-4)	0.09 ± 0.00 ^a	0.47 ± 0.02 ^b	0.45 ± 0.12 ^b	0.50 ± 0.00 ^b
8,11,14-Octadecatrienoic	18:3 (n-4)	3.86 ± 0.04 ^a	0.92 ± 0.12 ^b	1.19 ± 0.16 ^b	1.08 ± 0.08 ^b
Σ (n-4)		4.73 ± 0.05^a	3.73 ± 0.28^b	3.72 ± 0.20^b	3.89 ± 0.13^b
Palmitidonic	16:4 (ω-3)	0.35 ± 0.00	0.40 ± 0.09	0.47 ± 0.14	0.37 ± 0.02
α-Linolenic	18:3 (ω-3)	ND	0.02 ± 0.03	ND	ND
Stearidonic	18:4 (ω-3)	0.19 ± 0.04	ND	ND	ND
Dihomo-α-linolenic	20:3 (ω-3)	0.15 ± 0.02	1.25 ± 1.58	0.13 ± 0.01	0.13 ± 0.00
Eicosatetraenoic	20:4 (ω-3)	0.92 ± 0.06 ^a	0.42 ± 0.00 ^b	0.45 ± 0.02 ^b	0.44 ± 0.04 ^b
Eicosapentaenoic (EPA)	20:5 (ω-3)	6.91 ± 0.19	7.36 ± 0.68	7.32 ± 0.39	6.96 ± 0.08
Heneicosapentaenoic	21:5 (ω-3)	0.40 ± 0.06	0.47 ± 0.12	0.33 ± 0.03	0.40 ± 0.02
Docosapentaenoic (DPA)	22:5 (ω-3)	0.24 ± 0.09 ^a	1.42 ± 0.11 ^b	0.58 ± 0.04 ^c	1.04 ± 0.15 ^d
Docosahexaenoic (DHA)	22:6 (ω-3)	8.78 ± 0.66 ^a	17.02 ± 1.98 ^b	17.25 ± 0.48 ^b	16.63 ± 0.18 ^b
Σ (ω-3)		17.95 ± 0.54^a	28.36 ± 4.35^b	26.53 ± 0.21^b	25.97 ± 0.46^b
Linoleic	18:2 (ω-6)	1.52 ± 0.01 ^a	1.36 ± 0.00 ^b	1.43 ± 0.01 ^a	1.41 ± 0.11 ^a
γ-Linolenic	18:3 (ω-6)	1.35 ± 0.06 ^a	1.05 ± 0.05 ^a	1.08 ± 0.00 ^a	0.83 ± 0.22 ^b
Dihomo-linoleic	20:2 (ω-6)	0.25 ± 0.01	0.32 ± 0.07	0.28 ± 0.04	0.27 ± 0.00
Dihomo-γ-linolenic	20:3 (ω-6)	ND ^a	0.14 ± 0.04 ^b	0.12 ± 0.01 ^b	0.13 ± 0.01 ^b
Arachidonic	20:4 (ω-6)	0.41 ± 0.08	1.11 ± 1.39	1.87 ± 0.12	2.09 ± 0.12
Σ (ω-6)		3.54 ± 0.02	3.99 ± 1.33	4.78 ± 0.10	4.74 ± 0.24
Σ PUFA		26.22 ± 0.51^a	36.08 ± 2.75^b	35.04 ± 0.52^b	34.59 ± 0.11^b
ω-3/ω-6		5.07 ± 0.12	7.72 ± 3.36	5.55 ± 0.08	5.49 ± 0.37
PUFA/SFA		1.14 ± 0.03	1.02 ± 0.14	1.03 ± 0.00	0.97 ± 0.00
Polyene Index		1.16 ± 0.05	1.13 ± 0.21	1.17 ± 0.02	1.04 ± 0.00
Atherogenic index (AI)		0.54 ± 0.00 ^a	0.79 ± 0.08 ^b	0.76 ± 0.02 ^b	0.82 ± 0.00 ^b
Thrombogenic index (TI)		0.26 ± 0.01	0.32 ± 0.06	0.32 ± 0.00	0.35 ± 0.01

^{a, b, c, d, e} Means of with different superscripts within a row are significantly different at $\alpha < 0.05$. Σ = sum; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ND = not detected.

6.3.1.3 *Amino acid composition*

The total amino acids (Table 6.3) in the smoked samples were all significantly higher ($p < 0.05$) than that of the FM (i.e. 190.39, 397.36, 408.73 and 449.46 mg/g for FM, SM, SMg-1.5 kGy and SMg-3.0 kGy respectively). Irradiation increased the amino acid composition, however this was not statistically different ($p > 0.05$) from the SM samples or between the two doses (1.5 and 3.0 kGy). The essential amino acids (EAA) accounted for a greater proportion (about 55%) of the total, with FM significantly lower than SM, SMg-1.5 kGy and SM-3.0 kGy (106.31, 211.50, 228.98 and 252.82 mg/g respectively). With the exception of methionine, all other EAAs were significantly higher ($p < 0.05$) in the smoked samples than FM. Histidine concentration was significantly higher ($p < 0.05$) in SMg-3.0 kGy than SM (i.e. 25.57 and 15.84 mg/g respectively). The non-essential amino acids (NEAA) followed a similar pattern as the EAAs, with SMg-3.0 kGy having the highest proportion (78.57 mg/g) and FM having the lowest (33.43 mg/g). Serine and alanine were significantly higher ($p < 0.05$) in all smoked samples, with aspartic acid being significantly higher in the SMg samples relative to both FM and SM. The total conditionally indispensable (CI) amino acids were significantly higher in all smoked samples than FM, and ranged between 50.65 to 118.06 mg/g for FM and SMg-3.0 kGy respectively). Glycine and proline were significantly higher ($p < 0.05$) in all smoked samples, cysteine was only significantly higher ($p < 0.05$) in the SMg samples, whereas there was no significant difference in glutamine and arginine between the FM and smoked samples. The ratio of EAA to NEAA was not significantly different ($p > 0.05$) between all samples, and ranged from 1.35 to 1.45.

Table 6.3: Amino acid composition of fresh (F), smoked (S) and gamma irradiated (g) mackerel (M) (mg/g)

Amino acids	FM	SM	SMg-1.5 kGy	SMg-3.0 kGy
<i>Essential/Indispensable (EAA)</i>				
Threonine	9.23 ± 0.89 ^a	17.95 ± 1.08 ^b	18.10 ± 1.03 ^b	18.08 ± 1.90 ^b
Methionine	4.93 ± 1.10	9.45 ± 0.26	12.07 ± 1.28	11.87 ± 3.99
Valine	19.80 ± 1.00 ^a	41.67 ± 2.54 ^b	43.84 ± 2.32 ^b	49.38 ± 5.59 ^b
Histidine	7.06 ± 1.41 ^a	15.84 ± 1.02 ^{bc}	22.01 ± 2.25 ^b	25.57 ± 1.87 ^{bd}
Lysine	25.26 ± 0.36 ^a	46.52 ± 4.29 ^b	48.30 ± 1.11 ^b	51.92 ± 4.28 ^b
Leucine	13.27 ± 0.49 ^a	29.26 ± 1.10 ^b	27.48 ± 2.01 ^b	30.81 ± 2.52 ^b
Phenylalanine	7.36 ± 0.07 ^a	16.26 ± 0.49 ^b	14.97 ± 1.11 ^b	16.65 ± 0.19 ^b
Tryptophan	6.44 ± 0.39 ^a	12.98 ± 0.29 ^b	12.57 ± 0.35 ^b	13.82 ± 1.25 ^b
Isoleucine	12.96 ± 1.86 ^a	31.58 ± 0.07 ^b	29.65 ± 1.26 ^b	34.74 ± 3.20 ^b
Σ EAA	106.31 ± 4.03^a	211.50 ± 10.61^b	228.98 ± 7.94^b	252.82 ± 24.79^b
<i>Non-essential/Dispensable (NEAA)</i>				
Serine	7.58 ± 0.74 ^a	14.12 ± 0.31 ^b	15.40 ± 0.89 ^b	16.70 ± 2.19 ^b
Alanine	7.97 ± 0.39 ^a	17.99 ± 0.69 ^b	18.11 ± 0.48 ^b	19.67 ± 2.24 ^b
Aspartic acid	17.89 ± 0.63 ^a	35.78 ± 3.55 ^a	39.38 ± 3.31 ^b	42.20 ± 7.44 ^b
Σ NEAA	33.43 ± 1.72^a	67.90 ± 4.56^b	72.89 ± 4.69^b	78.57 ± 11.87^b
<i>Conditionally indispensable (CI)</i>				
Glycine	6.29 ± 0.42 ^a	15.63 ± 1.07 ^b	15.11 ± 0.25 ^b	17.52 ± 2.33 ^b
Proline	5.21 ± 0.55 ^a	12.63 ± 1.28 ^b	13.12 ± 1.02 ^b	14.18 ± 1.25 ^b
Glutamine	28.72 ± 2.32	52.60 ± 0.56	52.51 ± 11.80	57.30 ± 9.95
Cysteine	1.10 ± 0.29 ^a	2.32 ± 0.12 ^a	2.60 ± 0.31 ^b	2.67 ± 0.48 ^b
Arginine	9.33 ± 0.91	24.77 ± 6.25	23.51 ± 3.77	26.38 ± 5.91
Σ CI	50.65 ± 3.91^a	107.96 ± 9.28^b	106.86 ± 14.50^b	118.06 ± 19.92^b
Σ TAA	190.39 ± 9.66^a	397.36 ± 24.45^b	408.73 ± 27.12^b	449.46 ± 56.59^b
Σ EAA/Σ NEAA+CI	1.27 ± 0.04	1.26 ± 0.04	1.28 ± 0.09	1.29 ± 0.08

^{a, b}, Means of with different superscripts within a row are significantly different at $\alpha < 0.05$.

6.3.1.4 pH

The pH increased in all the smoked samples (not significantly though, $p > 0.05$) relative to FM (Figure 6.1). There was a general increase in pH during the refrigerated storage of the smoked mackerel, with that in SMg samples higher than SM. Samples stored at ambient temperature (SM-A, SMg-1.5-A and SMg-3.0-A) decreased by Day 5 of storage. Again, the changes between the different products, storage conditions and days were not statistically significant ($p > 0.05$).

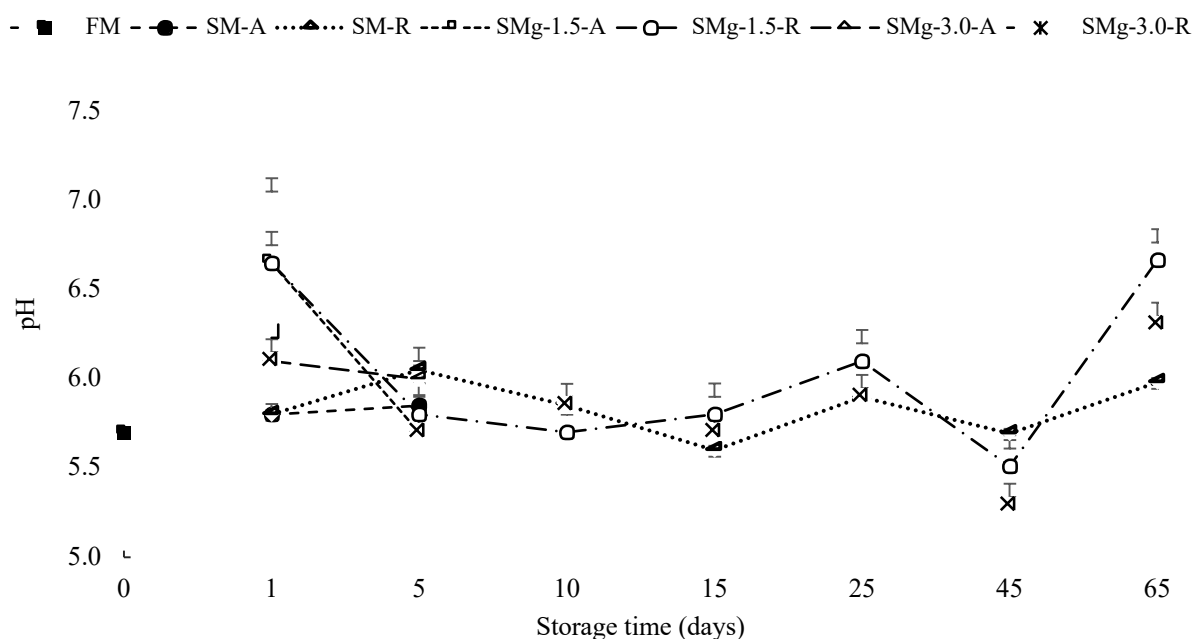


Figure 6.1: pH of fresh (F), smoked (S) and gamma irradiated (g) mackerel (M) stored at ambient (A) and refrigerated (R) temperatures for 65 days. Refrigerated (R = 2-4°C) and ambient temperatures (A = 27-30°C)

6.3.1.5 Total volatile base (TVB)

The total volatile base (TVB) increased significantly ($p < 0.05$) in all smoked samples (Day 1), compared to the FM (Figure 6.2). The SMg samples were however significantly lower ($p < 0.05$) than the SM ones on Day 1. By Day 5, the SMg samples stored at ambient temperature had increased TVB content (84.70 mg N/100g and 87.15 mg N/100g for SMg-1.5-A and SMg-3.0-A respectively), which were significantly higher ($p < 0.05$) than the SM samples. There was, however, a general decreasing trend in TVB during the rest of the storage period at

refrigerated temperature. This trend was however not significant different ($p > 0.05$) in terms of irradiation treatment and storage days.

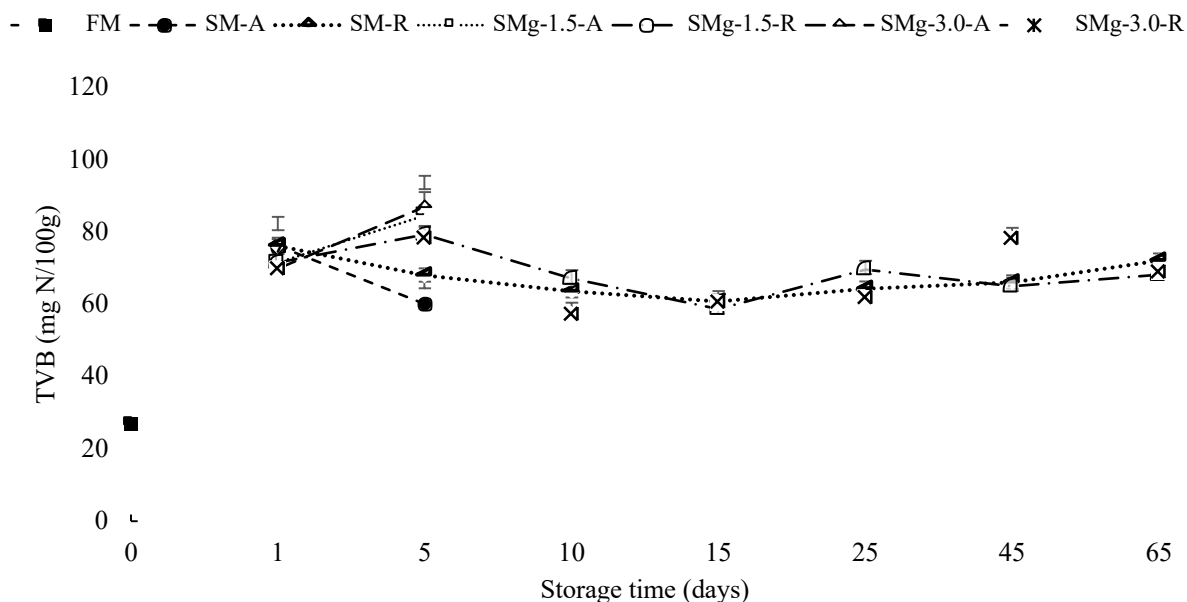


Figure 6.2: Total volatile base (TVB) content of fresh (F), smoked (S) and gamma irradiated (g) mackerel (M) stored at ambient (A) and refrigerated (R) temperatures for 65 days. Refrigerated (R = 2-4 °C) and ambient temperatures (A = 27-30 °C)

6.3.1.6 Peroxide value (PV)

The PVs for FM, SM, SMg-1.5 kGy and SM-3.0 kGy were 10.34, 10.43, 16.85 and 9.61 meq O₂/kg respectively (Figure 6.3), with SMg-1.5 kGy being significant higher ($p < 0.05$) than all the other samples. On Day 5, there was a reduction in PV for SM-A and SM-R and a significant increase ($p < 0.05$) in all SMg samples, relative to SM, irrespective of their storage temperature. SMg-1.5 kGy was also significantly higher ($p < 0.05$) under refrigeration, compared to ambient storage. There was a general increase in PV for all samples during storage at refrigerated temperature, in the order of SM < SMg-1.5 kGy < SMg-3.0 kGy. On Days 10 and 45, SM was significantly lower ($p < 0.05$) than both SMg samples, whereas SMg-3.0 kGy was significantly higher ($p < 0.05$) than SM (on Day 15) and also SM and SMg-1.5 kGy (on Day 25). On Day 65 however, there were no significant differences in the PV for all samples.

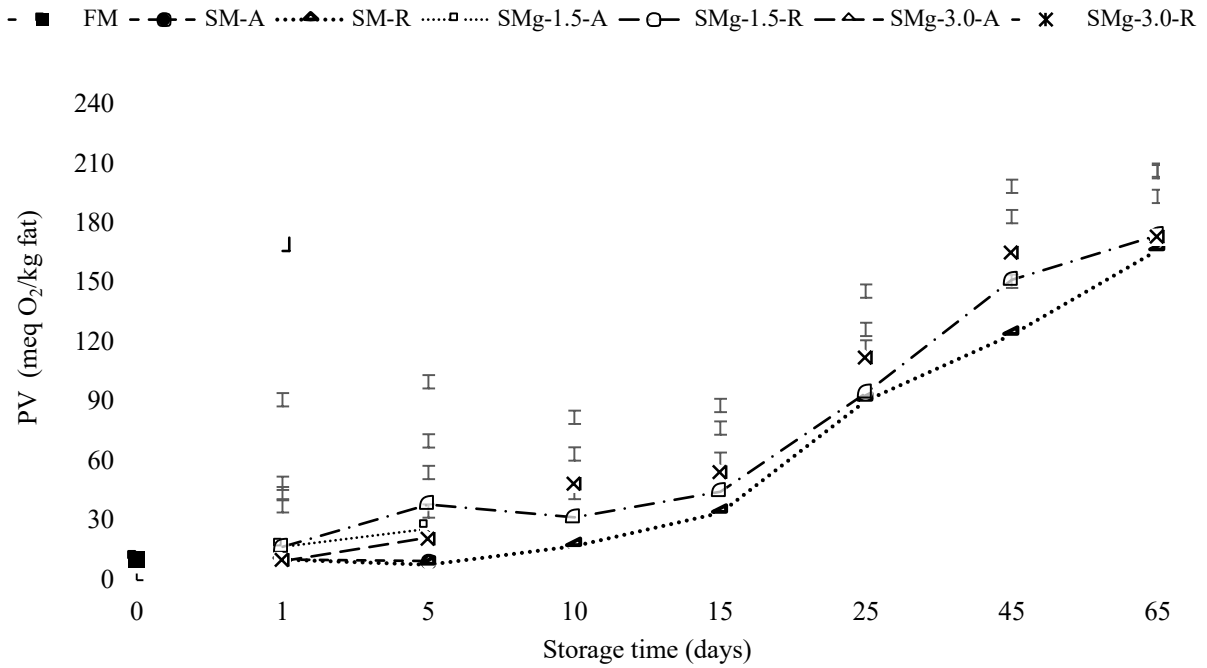


Figure 6.3: Peroxide value (PV) of fresh (F), smoked (S) and gamma irradiated (g) mackerel (M)

6.3.1.7 Free fatty acid (FFA)

Fresh mackerel had an FFA of 8.84 g/100g fat, which was significantly reduced ($p < 0.05$) after smoking and irradiation (Figure 6.4). By Day 5, SMg-3.0-A had the highest FFA content (7.8 g/100g fat), which was significantly higher ($p < 0.05$) than all the other samples, irrespective of their storage temperature. There is a general increase in FFA during storage, with SMg-1.5-R having a significantly higher ($p < 0.05$) level (9.15 g/100g) than SM-R and SMg-3.0-R (5.38 and 4.37 g/100g respectively) on Day 25, after which the levels decreased to 4.01, 4.69 and 4.07 g/100g (SM-R, SMg-1.5-R and SMg-3.0-R respectively) on Day 65.

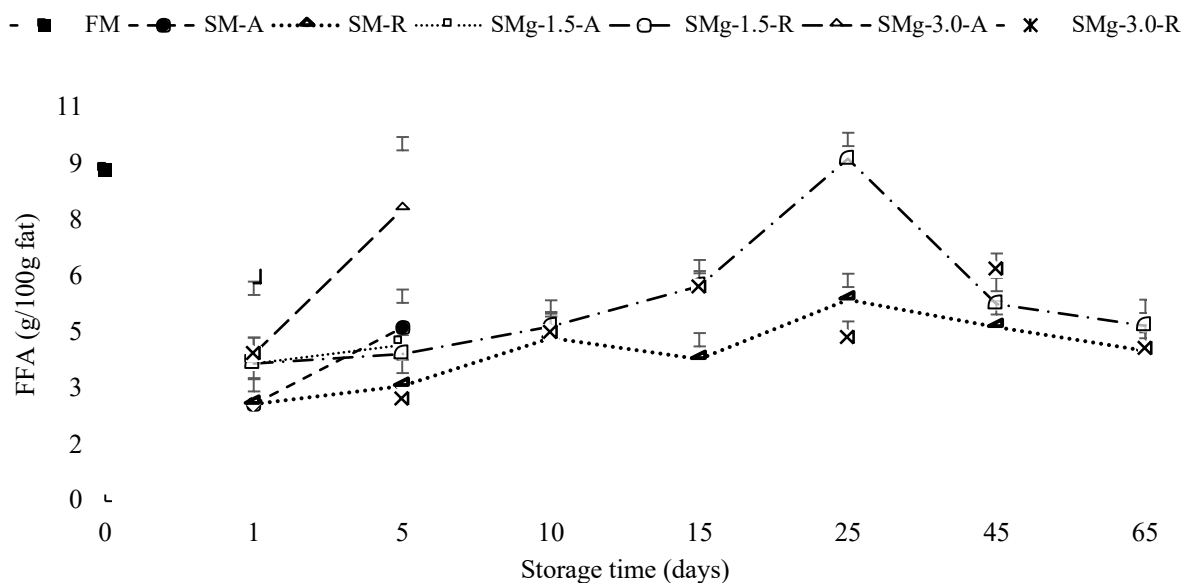


Figure 6.4: Free fatty acid (FFA) composition of fresh (F), smoked (S) and gamma irradiated (g) mackerel (M) stored at ambient (A) and refrigerated (R) temperatures for 65 days. Refrigerated (R = 2-4 °C) and ambient temperatures (A = 27-30°C)

6.3.2 Microbial analyses

The microbial quality of non-irradiated and irradiated smoked fish stored for 65 days is presented in Figure 6.5.

6.3.2.1 Total mesophilic count (TVC)

The TVC for the FM was 2.5 log CFU/g, and this increased in the irradiated samples on Day 1 (Figure 6.5a). On Day 5, the counts decreased in all samples, except for SM-A, which was significantly higher ($p < 0.05$). The counts in SMg-1.5-R were further significantly lower ($p < 0.05$) than in SM and SMg-3.0-A. From Day 10, there was a general increase in TVC for all samples with Day 65 recording counts of 2.2, 3.3 and 1.4 log CFU/g in SM-R, SMg-1.5-R and SMg-3.0-R respectively. The samples however did not significantly differ ($p > 0.05$) during this time.

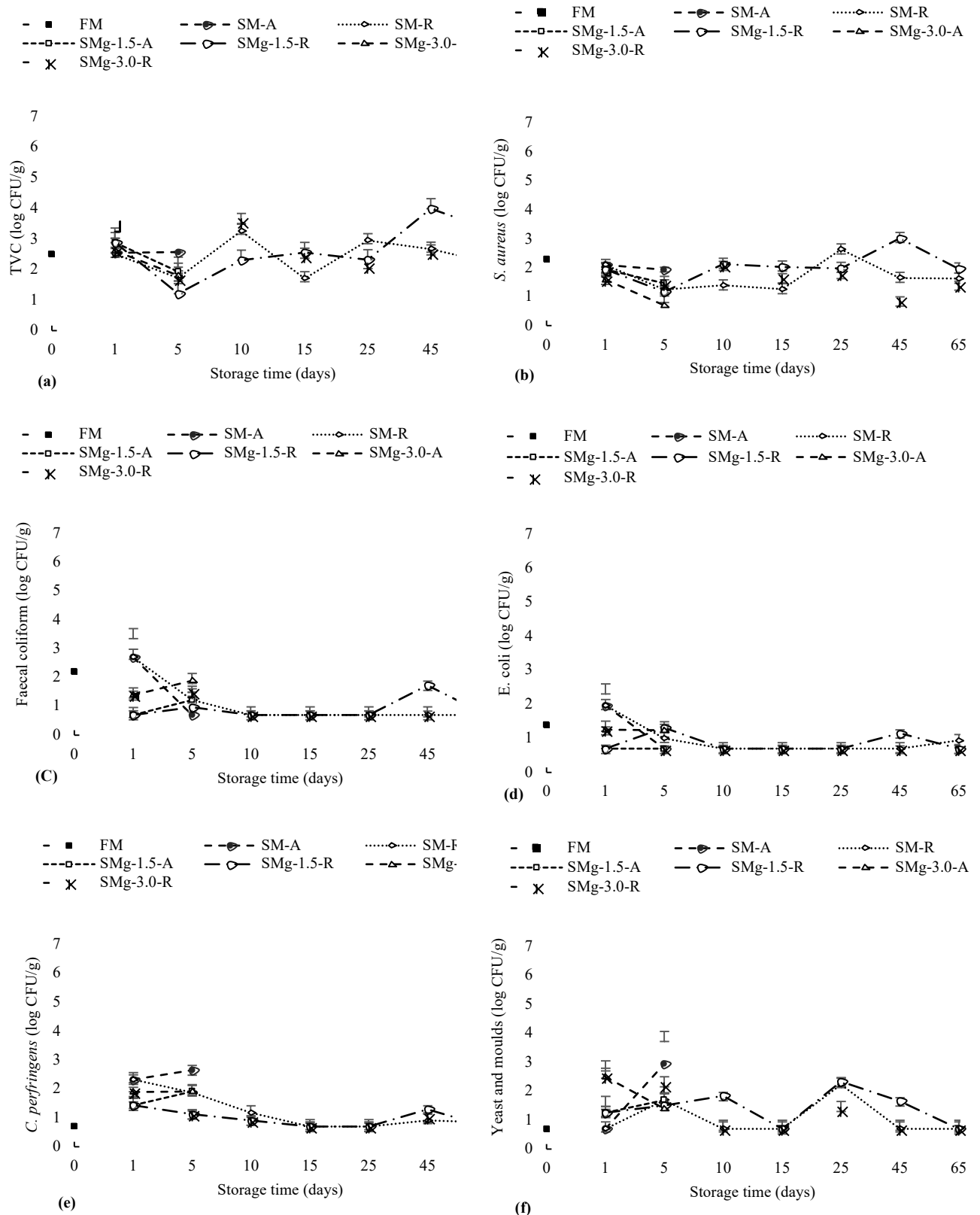


Figure 6.5: Total mesophilic (a), *S. aureus* (b), faecal coliform (c), *E. coli* (d), *C. perfringens* (e) and yeast and mould (f) counts in fresh (F), smoked (S) and gamma irradiated (g) mackerel (M) stored at ambient (A) and refrigerated (R) temperatures for 65 days. Refrigerated (R = 2-4 °C) and ambient temperatures (A = 27-30 °C)

6.3.2.2 *Faecal coliform and E. coli counts*

The faecal coliform and *E. coli* counts in FM were 2.2 and 1.4 log CFU/g respectively, which increased after smoking to 2.7 and 2.0 log CFU/g in SM on Day 1 (Figure 6.5b, c). Irradiation resulted in a significant decrease ($p < 0.05$) in SMg-1.5-A, relative to SM. The counts generally increased in all samples, except SM-A, on Day 5. The changes however were not statistically different ($p < 0.05$). From Day 10 to 65, faecal coliform and *E. coli* were below detectable limits in SM-3.0-R. SMg-1.5-R only recording counts on Day 45 (1.7 and 1.2 log CFU/g for faecal coliform and *E. coli* respectively), while SM-R had counts of 0.9 log CFU/g only on Day 65.

6.3.2.3 *Staphylococcus aureus*

S. aureus counts reduced after smoking (Figure 6.5d), from 2.3 log CFU/g in FM to 1.6 log CFU/g in SMg-3.0-A (which were significantly lower ($p < 0.05$) than the other smoked samples). There were further decreases by Day 5, with the irradiated samples being significantly lower than SM, at both refrigerated and ambient storage temperatures. *S. aureus* counts increased in all samples on Day 10, after which they generally increased. The highest count occurred on Day 45 in SMg-1.5-R (3.0 log CFU/g), which was significantly higher than SM-R and SMg-3.0-R.

6.3.2.4 *Clostridium perfringens*

C. perfringens were below detectable limits in FM but increased in SM to 2.3 log CFU/g on Day 1 (Figure 6.5e). Irradiation further reduced the counts, with SMg-1.5-A being significantly lower. The counts increased in all samples stored at ambient temperature, but the irradiated samples were significantly lower than SM, on Day 5. The refrigerated samples had reduced counts, with SMg-3.0-R having significantly lower ($p < 0.05$) counts. The counts generally

decreased during storage, with Days 15, 25 and 65 having counts below detection limits in all irradiated samples.

6.3.2.5 Yeast and moulds

Yeast and moulds were also below detection limits in FM and SM samples (Figure 6.5f). Irradiated samples had increased counts, i.e. 1.3 and 2.5 log CFU/g in SMg-1.5 and SMg-3.0 respectively. Moulds were visually observed in smoked samples stored at ambient temperature on Day 5. Laboratory analysis estimated increased counts for SM and SMg-1.5 and decreased counts in SMg-3.0, though these were not statistically different ($p > 0.05$). The counts in SMg-1.5-R were significantly higher ($p < 0.05$) on Days 10 and 45 (1.8 and 1.7 log CFU/g respectively), while SMg-3.0-R had significantly higher counts on Day 25. All samples had counts below detectable limits on Day 65.

6.3.3 Insect infestation

Visual inspection of the samples on Day 5 showed insect larvae in all samples, both irradiated and non-irradiated, stored at ambient temperature.

6.3.4 Colour analysis

The instrumental skin colour measured showed irradiation increased the L^* and b^* from 34.45 to 43.91 and 5.93 to 11.66 in SM and SMg-3.0 kGy respectively (Table 6.4). The a^* was also highest in SMg-1.5 kGy. The H^o_{ab} and c^* were also highest in the SMg-3.0 kGy samples. Similar results were obtained for the muscle colour. These differences in skin and muscle colour between the SM and irradiated samples were however not significantly different ($p > 0.05$).

Table 6.4: Skin and muscle colour characteristics of smoked (S) and gamma irradiated (g) mackerel (M)

Parameter	SM	SMg-1.5 kGy	SMg-3.0 kGy	
Skin	Lightness (L^*)	34.45±1.31	39.62±2.62	43.91±10.15
	Redness (a^*)	-0.62±0.66	0.31±2.16	-1.10±0.19
	Yellowness (b^*)	5.93±5.16	5.24±0.88	11.66±8.20
	Hue angle (H^o_{ab})	76.77±17.43	73.94±1.80	82.50±6.15
	Chromaticity (c^*)	6.06±5.00	5.46±0.96	11.74±8.13
Muscle	Lightness (L^*)	60.56±12.25	66.61±6.75	66.62±0.86
	Redness (a^*)	2.66±2.49	3.96±1.24	1.91±0.47
	Yellowness (b^*)	20.03±0.95	17.28±2.13	20.66±1.05
	Hue angle (H^o_{ab})	82.33±7.33	76.83±5.46	84.76±1.03
	Chromaticity (c^*)	20.29±0.61	17.77±1.80	20.75±1.10

6.3.5 Sensory analysis

The mean scores for SM, SMg-1.5 kGy and SMg-3.0 kGy were 2.3, 2.3 and 2.6 respectively, on the 5-point scale (Figure 6.6). These were not statistically different ($p > 0.05$). The results from the Check-all-that-apply (CATA) (Figure 6.7), showed important attributes that may have accounted for the perceived differences between the fish treatments and the control sample were juicy texture, umami flavour, smoked flavour and aroma and herring-like aroma. Other attributes related to the saltiness (SMg-1.5 kGy and SMg-3.0 kGy saltier and slightly softer than SM).

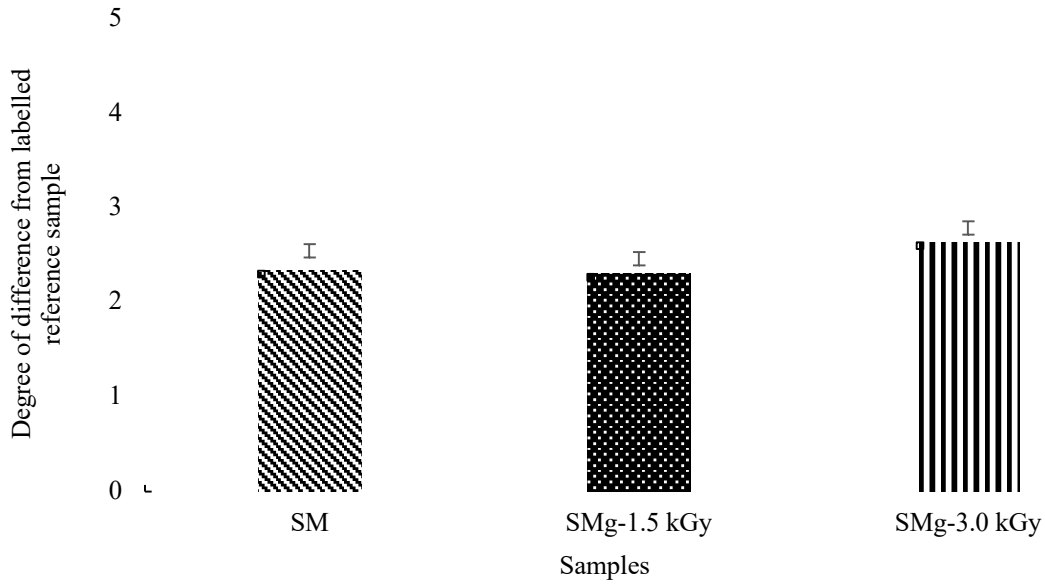


Figure 6.6: Mean intensity scores for difference-from-control test (Scale: 0 = No difference; 1= Very slight difference; 2 = Slight difference; 3 = Moderate; 4 = Very different and 5 = Extreme difference)

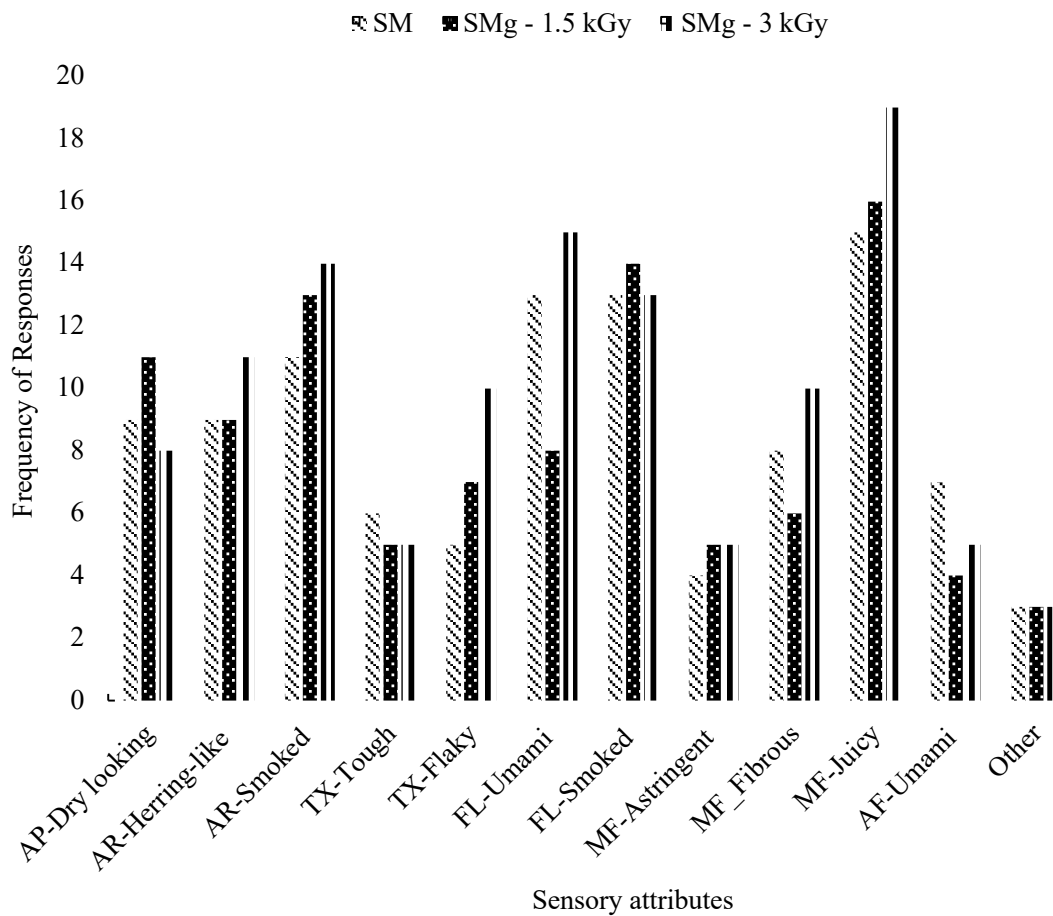


Figure 6.7: Frequency of responses for CATA attributes indicating perceived differences (AP- = Appearance; AR- = Aroma; TX- = Texture; FL- = Flavour; MF- = Mouthfeel; AF- = Aftereffect)

6.4 Discussion

The moisture content of SM and SMg samples were significantly reduced (compared to FM) and met the industrially specified limit ($< 65\%$) for 'smoked finished products', which was consistent with Cardinal et al. (2001) and Asamoah (2018). The protein content was also significantly increased in the smoked samples relative to FM. However, there were no significant differences in the moisture, protein, fat, ash and pH between the SM and SMg mackerel. The sensory and colour analyses also found no statistical difference between the SM and SMg samples. This was in consonance with a study by Badr (2012) on the effect of irradiation on cold-smoked salmon that found that the proximate composition and sensory acceptability were not affected by doses up to 3 kGy. A similar study by Dvorak, Kratochv, & Grolichov (2005) found no differences in colour and pH of *Onchorynchus mykiss* irradiated at a dose of 3 kGy. The proximate composition of the SM and SMg samples did not significantly change during the 65 days of storage. This could have been as a result of the refrigerated storage, since Al-Reza, et al. (2015) reported protein and fat degradation in sun-dried fish stored at ambient temperature for 60 days. Silva, Mendes, Nunes, & Empis (2006) further indicated that irradiation up to 10 kGy had no significant impact on proteins in horse mackerel stored in ice.

Smoking resulted in a significant increase in SFA, MUFA and PUFA, which could be due to the loss of water via evaporation during the smoking process (Bouriga, Bejaoui, Jemmali, Quignard, & Trabelsi, 2020). The proportions of fatty acids in FM was of the order MUFA $>$ PUFA $>$ SFA, similar to findings by Nogueira, Cordeiro, & Aveiro (2013). In contrast, the SM and SMg samples were of the order PUFA \approx SFA $>$ MUFA, which was not consistent with results from Cyprian (2015), Chaula, et al. (2019) and Erkan & Özden (2007). The individual fatty acids that contributed the greatest proportions in all samples were palmitic, myristic and

stearic acids (SFAs), oleic acid (MUFA) and eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (PUFAs). Similar findings were reported for irradiated sea breams (Erkan & Özden, 2007), Atlantic chub mackerel and other species captured in north-eastern Atlantic (Nogueira, Cordeiro, & Aveiro, 2013), golden grey mullet and gold band goatfish (Küçükgülmez, Yanar, Çelik, & Ersor, 2018) and tilapia (Surendra, Edirisinghe, & Rathnayake, 2018). Oleic acid is important in human nutrition as it stimulates bile secretion, which aids in the digestion and absorption of fats (Nogueira, Cordeiro, & Aveiro, 2013). Again, EPA and DHA are also hypotriglyceridemic and very important in the prevention of cardiovascular and inflammatory diseases in humans (Nogueira, Cordeiro, & Aveiro, 2013; Cyprian, 2015). The high levels of EPA and DHA in FM, SM and SMg implied that they were of good nutritional quality.

There was a higher proportion of ω -3 PUFAs compared to ω -6, which agrees with the assertion that marine fish are richer in ω -3 than ω -6 (Osman, Suriah, & Law, 2001). The ω -3: ω -6 ratio is a good index for comparing the relative nutritional value of fish (Küçükgülmez, Yanar, Çelik, & Ersor, 2018). The ratios from the present study were between 5.07 and 7.72 (FM and SM respectively), and these were within the estimates of 2.67 to 12.61 in marine species (Bayir, Haliloglu, Sirkecioglu, & Aras, 2006). Küçükgülmez, Yanar, Çelik, & Ersor (2018) stated that higher ratios (>1) are of great importance in order as this diminishes the risks of coronary heart diseases, plasma lipid levels, and cancer in humans.

Lipid quality was assessed using three indices: the polyene index (PI), index of atherogenicity (AI) and index of thrombogenicity (TI). The PI measures oxidative damage to PUFAs (Rodríguez, et al., 2007). Consuming foods rich in fatty acids can directly affect the simulation or preclusion of atherosclerosis and coronary thrombosis due to their effect on blood

cholesterol and low-density lipoprotein (LDL) cholesterol concentrations (Omri, et al., 2019). According to Garaffo, et al. (2011), the index of atherogenicity (AI) and index of thrombogenicity (TI) can be used to characterise these effects. The AI denotes the relationship between the main pro-atherogenic (saturated fatty acids that favour the adhesion of lipids to cells of the immunological and circulatory system) and anti-atherogenic (unsaturated fatty acids that inhibit the aggregation of plaque and diminishes the levels of esterified fatty acid, cholesterol, and phospholipids, there-by preventing the appearance of micro- and macro-coronary diseases) fatty acids. The index of thrombogenicity (TI) defines the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFAs, ω -6 PUFAs and ω -3 PUFAs) that have the tendency to form clots in blood vessels (Garaffo, et al., 2011). According to Łuczyńska, Paszczyk, Nowosad, & Łuczyński (2017), AI and TI greater than 1.0 can be detrimental to human health. The results from the present study show AI and TI values below 1.0 (with FM having the lowest estimates), indicating there are reduced risks of atherosclerosis and coronary thrombosis from consuming these products. These estimates were however higher than those for fresh mackerel, shrimp and lobster (Rosa & Nunes, 2003), lower than estimates in raw, frozen, solar-dried and oven-dried sardinella (Telahigue, Hajji, Rabeh, & El Cafsi, 2013), but similar to those for bluefin tuna roes and salted 'Bottarga' (Garaffo, et al., 2011). Küçükgülmez, Yanar, Çelik, & Ersor (2018) observed seasonal variations in AI, TI, and TI of gold band goatfish and golden grey mullet from the Mediterranean seas. The PUFA/SFA ratios calculated in the present study were about 1 (between 0.97 to 1.14, FM and SMg-3.0 kGy respectively). Regulska-Ilow, et al. (2013) stated that a ratio ≥ 1 could decrease the possibility of atherosclerosis and coronary heart disease, which was consistent with the results of this study. Irradiation at 1.5 and 3.0 kGy did not have a significant effect on the fatty acid composition of smoked fish, which agrees with assertions by (Erkan & Özden, 2007).

Amino acids form the molecular structure of proteins, responsible for the synthesis of hormones, enzymes, body tissues and other metabolic molecules (Salma & Nizar, 2015). They are classified as essential (EAA, i.e. indispensable as they cannot be synthesized *de novo*, and therefore must be supplied by diet); non-essential (NEAA, i.e. dispensable as they synthesised by the body) and conditionally indispensable (CI, as they are normally synthesised by the body but can be limited under special pathophysiological conditions) (Otten, Hellwig, & Meyers, 2006). A total of 17 amino acids (made up of 9 EAA, 3 NEAA and 5 CI) were identified in this study. The presence of the 9 EAA makes fish protein a 'complete protein', as was reported by Otten, Hellwig, & Meyers (2006). The total amino acid content in FM, SM and SMg-1.5 kGy and Smg-3.0 kGy were 190.39, 397.36, 408.73 and 449.46 respectively. The dominant amino acids were valine, lysine, leucine and isoleucine (EAA); aspartic acid (NEAA); and glutamine (CI), which was comparable with findings by Rosa & Nunes (2003), Erkan & Özden (2007) and Atowa, Nwabu, & Ogiedu (2014). The benefits of amino acids in human nutrition have been examined by several authors. Mohanty, et al. (2014) reported that aspartic acid, methionine arginine and glycine play an important role in wound healing and also in maintaining the solubility and ionic properties of proteins. Sarma, et al. (2013) further reports that methionine, histidine, lysine and tryptophan have antioxidant properties. Again, Kim, et al. (1999) stated that glutamine, proline, aspartic acid, glycine and leucine have cytotoxic abilities to kill or damage cancer cells. Irradiation at 1.5 and 3.0 kGy did not significantly affect these amino acids, meaning that the products i.e. FM, SM and SMg, were all of very good nutritional status.

Amino acids can also be used as quality indices in seafood. Histidine, tyrosine, arginine, tryptamine and lysine are very important during fish spoilage, as they can produce biogenic amines (via decarboxylation by microorganisms), like histamine, tyramine, agmatine,

tryptophan and cadaverine respectively (Biji, Ravishankar, Venkateswarlu, Mohan, & Gopal, 2016). From the results, the fresh and smoked samples had appreciable levels of these amino acids and hence care should be taken to ensure that microbial activities are diminished during storage to inhibit biogenic amine formation. Again, glutamic acid, aspartic acid, alanine and glycine, are responsible for characteristic flavor and taste of fish Erkan & Özden (2007). The levels of glycine and alanine were not statistically different between the SM and SMg samples. Only aspartic acid was significantly higher in SMg samples compared to SM but this was probably not sufficient to change the flavour and taste of the irradiated samples, as evidenced by the results from sensory analysis. Higher irradiation doses however can change the flavour and taste in foods, as reported by Erkan & Özden (2007). Smoking has however been shown to reduce the lysine content in foods (as they react with carbonyls in smoke during browning or Maillard reaction) (Kaya, Turan, & Erdem, 2008). The result from the present study however contradicts this assertion, as lysine levels significantly increased in the smoked products.

The ratio of EAA to NEAA highlights the important contributions of each to the diet. From the present study, the ratios were 1.27, 1.26, 1.28 and 1.29 for FM, SM, SMg-1.5 kGy and SMg-3.0 kGy respectively. These ratios were higher than those reported by Rosa & Nunes (2003), Salma & Nizar (2015) and Kaya, Erdem, & Turan (2014). It can therefore be inferred that fresh, smoked and irradiated (at 1.5 and 3.0 kGy) mackerel are important sources of essential amino acids.

The total volatile base (TVB) is a measure of the fitness for consumption of fish products (Asamoah, 2018). The limits for fresh and smoked/dried fish are 25-30 mg N/100g and 100-200 mg N/100g (Özoğul & Özoğul, 2000; EU Directive, 2008). From the results, FM met this requirement and as such was fit for smoking and consumption. There was a significant increase

in TVB after smoking, which Goulas & Kontominas (2005) attributed to the partial drying and subsequent concentration of TVB contents in the smoked products. The levels of TVB in the non-irradiated and irradiated smoked mackerel were below 100 mg N/100g, even by Day 65 of storage at refrigerated temperature and Day 5 of ambient storage. This gave an indication that the products were fit for human consumption. Özoğul & Özoğul (2000) reported that TVB was not a reliable indicator of early quality changes as levels usually increase appreciably during the later stages of storage as a result of bacterial activity. Plahar, Nerquaye-Tetteh, & Annan (1999), in a study in Ghana obtained higher TVB levels of 120.6 and 133.8 mg/100g in freshly smoked *Sardinella* and anchovy respectively and 108.8 mg N/100g and 294.8 mg N/100g in the products stored for 6 months at ambient temperatures.

The peroxide value (PV) measures primary oxidation of lipids in foods (Salaudeen, 2013). PV values between 20-40 meq O₂/kg may indicate oxidation, and thus, developing spoilage (Daramola, Fasakin, & Adeparusi, 2007). The PV of FM was below this guideline, indicating it was of good quality for smoking. Smoking did not significantly increase the PV, which, could mean that the antioxidant properties of smoke may have exceeded the effects of hot smoking temperature on lipid oxidation (Marc, Kaakeh, & Mbofung, 1998; Cyprian, 2015). There was a general increase in PV during storage, which was more pronounced in irradiated samples than in non-irradiated ones, that was in agreement with results from Surendra, Edirisinghe, & Rathnayake (2018). Irradiation-induced lipid peroxidation has been shown to be dose-dependent, and can be sped up as a result of the formation of lipid-free radicals, which under aerobic conditions can break down hydroperoxides and destroy antioxidants (Fan & Sommers, 2013). Irrespective of the storage temperature, the PVs were however below the suggested guideline, up till Day 10, 15 and 25 for SMg-3.0 kGy, SMg-1.5 kGy and SM respectively, implying a delay in oxidation, with respect to the dose rate. Gecgel (2013) also obtained a positive

correlation between the increase in PV, the dose applied and storage duration for irradiated meatballs. Ahn & Love (1999) obtained an increase in oxidation in irradiated pork products (immediately after irradiation), relative to non-irradiated ones but this difference disappeared during storage, which was contrary to results from the present study. Cozzo-Siqueira, Oetterer, & Gallo (2003) however found that oxidation was higher in irradiated tilapia and Spanish mackerel, when doses were higher than 10 kGy and fish was stored under low refrigeration.

Free fatty acid (FFA) is a tertiary product of rancidity and is produced by the hydrolysis of fish fats and oils, through lipase action (Cyprian, et al., 2017; Chaula, et al., 2019). According to Daramola, Fasakin, & Adeparusi (2007), rancidity of fish oils is usually noticeable when FFA is between 0.5-1.5 g/100g. From the results, all samples (fresh, non-irradiated and irradiated) had FFA values above this limit (with FM having the highest FFA), which could mean they had undergone lipase action. Smoking produced a significant reduction in FFA, which could have resulted from the hot smoking temperatures denaturing most enzymes that catalyse lipid hydrolysis and thereby liberating FFA (Cyprian, et al., 2017). The FFA levels increased during storage, with SM samples generally lower than the SMg samples (significantly so with SMg-1.5 kGy on Day 25). This could mean that irradiation at the two doses may have accelerated the hydrolysis of glycerol-fatty acid esters, which in turn liberated the FFA (Cyprian, 2015)

Fresh or processed seafood can be contaminated by microorganisms during processing or through cross-contamination from regular handling and packaging (Ehlermann, 2016). This can affect the quality and shelf life of seafood. The recommended levels of total mesophilic bacteria, faecal coliform, *E. coli*, yeast and mould and *S. aureus* (for human consumption) have been established by GSA (2013; 2019) as 7, 2, 3, 4 and 4 log CFU/g respectively for fresh and smoked fish in Ghana. That for *C. perfringens* is 4 log CFU/g (Center for Food Safety, 2014).

From the results, FM and SM, SMg-1.5 kGy and SMg-3.0 kGy (irrespective of their storage temperatures) were within these limits during the study period, except for faecal coliform counts in FM, SM-A and SM-R (on Day 1) and SMg-3.0 kGy (on Day 5). Smoking alone was not enough to reduce the counts of faecal coliforms, as these may be thermo-tolerant (Marc, et al., 2014). Irradiation however reduced these counts, but doses of 4.0 kGy and above have been known to completely eliminate coliforms in smoked salmon (WHO, 2000).

In terms of shelf life, the ambient-stored SM and SMg samples had a shelf life of 5 days, due to insect infestation and visible mouldiness. Similar findings were reported by (Plahar & Amevor, 2003) for smoked sardines stored at ambient temperatures. The authors, therefore, recommended the use of heat-sealed low-density polythene bags and frozen storage to extend shelf life. Non-irradiated and irradiated smoked samples stored at refrigerated storage were of good microbiological, chemical (TVB and pH) and nutritional quality by Day 65 (9 weeks), even though oxidation was evident (from FFA and PV results). Moini, et al. (2009) reported that irradiation at 3 kGy could control the microbial, protein and lipid oxidation in fresh rainbow trout stored at refrigerated temperature for up to 4 weeks. Duah, Emi-Reynolds, Kumah, & Larbi (2018) reported shelf lives of 5 and 9 weeks for non-irradiated and irradiated (2.5-10 kGy) smoke-dried anchovies, stored in polyethylene bags at ambient temperature. The authors recorded no insect infestation in the irradiated samples within the 9 weeks of storage. Aworh, Okparanta, & Oyedokun (2002) also obtained a shelf life of 4-6 months for irradiated (up to 6 kGy) smoke-dried catfish stored at ambient temperatures.

6.5 Summary of findings

The effect of irradiation at two doses (1.5 and 3 kGy) and storage temperature (ambient, 27-30°C and refrigerated, 2-4°C) on the quality and shelf life of smoke mackerel was investigated.

Irradiation at 1.5 and 3 kGy had no impact on the nutritional composition (protein, fat, fatty acid and amino acid compositions), sensory and colour characteristics of the smoked mackerel. With respect to storage, the non-irradiated and irradiated, kept at ambient temperature had a shelf life of 5 days, as a result of insect infestation and visible mouldiness. With refrigeration, the products were of good quality by Day 65, in terms of their microbiological and nutritional status. Lipid oxidation and hydrolysis however increased during storage, and were more pronounced in the irradiated mackerel. This study therefore indicates that fish smoking, with or without irradiation (at 1.5 and 3 kGy), combined with refrigeration is a very viable way to preserve fish even beyond 65 days.

CHAPTER SEVEN

7.0 CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

Three kilns, the Chorkor, Cabin and AGFS kilns representing uncontrolled, semi-controlled and controlled smoking technologies, were investigated based on their efficiency. Further, the Chorkor and Cabin were assessed based on the amount of flue gases produced. The yield was better in the Cabin and Chorkor but the fuel consumption/kg of smoked fish and processing rate were better in the AGFS. The firewood-operated kilns produced flue gases containing CO and PM well above Ghana's EPA emissions standard. For SO₂, NO_x however, the amount produced was below the emission standard and therefore it stands to reason that with better heat management, the firewood consumption, CO and PM emission could be decreased. This would help safeguard the health of the predominately female population who use these kilns and their environments. The Cabin could be a better alternative to the Chorkor since it performed better and was less expensive than the AGFS. However, the environmental benefits of the AGFS (use of LPG which meant deforestation would not be a problem) and high throughput capacity meant it could be the best alternative of the three.

Smoking improved the physical, chemical, microbiological and sensory quality of mackerel and barracuda. Only colour and sensory analysis could statistically differentiate between the products from the two different smoking kilns. The Cabin-smoked products had the more traditional qualities of smoked fish (appearance, odour and flavour) that the gas-smoked products lacked, probably because of the indirect smoke generation and/or short contact between the fish and the smoke in the AGFS.

The smoking process produced some carcinogenic substances such as PAHs and heavy metals in the processed fish. The fresh mackerel and barracuda were of good quality for smoking and posed minimal carcinogenic risks to consumers. With respect to the kilns, AGFS performed best, by producing smoked products with benzo(a)pyrene and PAH4 concentrations below the EU MLs (2 and 12 µg/kg respectively). The Cabin also produced smoked mackerel with 77% and 59% lower benzo(a)pyrene and PAH4 (only in Esa smoked fish) than the Chorkor. The levels of benzo(a)pyrene and PAH4 were however greater than the EU's MLs in all Chorkor and Cabin smoked samples (except for benzo(a)pyrene in E-CSM and E-CSB). The potential carcinogenic risks were of least concern in the gas smoked and all barracuda samples, moderate in the Cabin smoked mackerel and high in the Chorkor smoked mackerel. It could therefore be inferred that the presence of PAHs in the smoked fish was due to the type of kiln, smoking method (direct and indirect) and fuel used (LPG and firewood) for the treatments. Again, the magnitude of the carcinogenic risks depended largely on the fish ingestion rate, with higher benzo(a)pyrene and PAH4 levels not always corresponding to increased risks (as shown in the barracuda). Heavy metal (Hg, Pb and Cd) contamination was negligible in fresh and smoked mackerel and barracuda. This indicated that the smoke emitted might not have contained significant amounts of heavy metals and thus contamination was avoided. With increased consumption of smoked products however, these risks are likely to increase thereby necessitating the need for the AGFS and the use of alternate sources of fuel in the Cabin.

The effect of irradiation at two doses (1.5 and 3 kGy) and storage temperature (ambient, 27-30°C and refrigerated, 2-4°C) on the quality and shelf life of smoke mackerel was investigated. Irradiation at 1.5 and 3 kGy had no impact on the nutritional composition (protein, fat, fatty acid and amino acid compositions), sensory and colour characteristics of the smoked mackerel. With respect to storage, the non-irradiated and irradiated samples, kept at ambient temperature

had a shelf life of 5 days, as a result of insect infestation and visible mouldiness. With refrigeration, the products were of good quality by Day 65, in terms of their microbiological and nutritional status, though lipid oxidation was detectable. This study therefore indicates that fish smoking, with or without irradiation (at 1.5 and 3 kGy), combined with refrigeration is a very viable way to preserve fish even beyond 65 days.

7.2 Recommendations

The following recommendations are being made based on the findings from this research:

7.2.1 Policy

1. The Ministry of Fisheries and Aquaculture Development (MOFAD), National Fish Processors and Traders Association (NAFPTA) and non-governmental organisations (NGOs) should adopt and popularise the two smoking kilns. The cost of construction of the kilns could be subsidised to make them more affordable and attractive to processors.
2. Fish processors should be trained in healthy fish handling, processing and storage and also on the use and maintenance of the kilns.

7.2.2 Research

1. To ascertain if the differences between the Cabin smoked and gas smoked samples would be acceptable, a consumer study should be undertaken. The results would help in the education (where necessary) and commercialisation of the products.

2. There should be further PAH studies of smoke-dried fish using other sources of fuel in the Cabin and AGFS. There should also be regular data collection and testing of the samples from these kilns to ensure that they adhere to good manufacturing practices.

3. The potential of irradiation on smoked fish using different packaging materials and longer shelf life study should be explored for fresh, and other processed seafoods in Ghana. Consumer education on the benefits of irradiation should also be carried out.

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