Concern for man and his fate must always form the chief interest of all technical endeavours. Never forget this in the midst of your diagrams and equations.

- Albert Einstein -

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This thesis is dedicated to the memory of

Mrs. Oumou Khairy Ndiaye
[*who was the innovator of the FAO-Thiaroye Processing Technique*]

and

Mrs. Frieda Oduro
[*who was a Ghanaian fisheries post-harvest specialist and champion*]

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THE SHIFT FROM TRADITIONAL TO AN IMPROVED FISH SMOKING OVEN IN GHANA: IMPLICATIONS FOR FOOD SAFETY AND PUBLIC HEALTH

MPhil. Kennedy Bomfeh

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Bioscience Engineering

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Above all, I am grateful to God who made me, saved me, and granted me this opportunity to study a minute part of the vast intelligible universe He created. As C.S. Lewis quipped, *“[Humans] became scientific because they expected law in nature, and they expected law in nature because they believed in a Law Giver.”*

Kennedy Bomfeh
November 15, 2020
Ledeberg, Gent, Belgium

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOCS	American Oil Chemists Society
BaP	Benzo(a)pyrene
BMDL	Benchmark dose level
BoG	Bank of Ghana
CAC	Codex Alimentarius Commission
CATA	Check-all-that-apply
CFU	Colony forming unit
CNFTPA	Centre National de Formation des Techniciens des Pêches et Aquaculture (National Training Centre for Fisheries and Aquaculture Technicians)
Cs	Concentration score
CSIR	Centre for Scientific and Industrial Research, Ghana
DHA	Docosahexaenoic acid
EC	European Commission
EC	European Commission
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
EU	European Union
FAME	Fatty acid methyl ester
FAO	The Food and Agriculture Organization of the United Nations

FAO-RAF	Food and Agriculture organization of the United Nations Regional Office for Africa
FDA	Food and Drugs Authority, Ghana
FERG	Foodborne Disease Burden Epidemiology Reference Group
FRI	Food Research Institute, Ghana
FTT	FAO-Thiaroye Processing Technique
GC-FID	Gas Chromatograph-Flame Ionisation Detector
GC-MS	Gas Chromatograph-Mass Spectrometer
GDP	Gross Domestic Product
GEPC	Ghana Export Promotion Council
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
GSA	Ghana Standards Authority
GSS	Ghana Statistical Service
HACCP	Hazard Analysis Critical Control Point
HPLC	High Performance Liquid Chromatography
Hs	Hazard score
IAFI	International Association of Fish Inspectors
IARC	International Association for Research on Cancer
IRT	Institut de Recherche Technologique (Technological Research Institute)
IS	Internal standard
JECFA	The Joint FAO/WHO Expert Committee on Food Additives

LMIC	Low- and middle-income countries
MCDA	Multicriteria decision analysis
MoE	Margin of exposure
MoFAD	Ministry of Food and Aquaculture Development, Ghana
MUFA	Monounsaturated fatty acid
NAFPTA	National Fish Processors and Traders Association (Ghana)
NTE	Non-traditional export
PAH	Polycyclic aromatic hydrocarbons
Ps	Prevalence score
PUFA	Polyunsaturated fatty acids
QDA	Quantitative descriptive analysis
RASFF	Rapid Alert System for Food and Feed
SCF	Scientific Committee on Food
SDG	Sustainable Development Goals of the United Nations
SFP	Scombroid food poisoning
SNV	Stichting Nederlandse Vrijwilligers (Foundation of Netherlands Volunteers)
SPE	Solid phase extraction
SPSS	Statistical Pack for Social Sciences
tBME	Tert-butyl methyl ether
UG	University of Ghana
UHPLC	Ultra High Pressure Liquid Chromatography

UN	United Nations
USA	United States of America
USFDA	United States Food and Drugs Administration
WHO	World Health Organisation

TABLE OF CONTENTS

SUMMARY

Smoked fish is an important source of animal protein in Ghana. It is produced by a traditional processing method using traditional ovens (namely, the Chorkor smoker and the metal drum oven) that predispose the smoked products to high polycyclic aromatic hydrocarbon (PAH) contamination. This compromises the safety of the products, since PAHs are carcinogenic food safety hazards. A new oven – called the FAO-Thiaroye Processing Technique (FTT) – has been introduced in the country to address the problem. This study evaluated the food safety and public health implications of transitioning from the traditional ovens to the FTT.

In **Chapter 1**, available literature on the importance of smoked fish and fish smoking to food and nutrition security and livelihood support in Africa in general and Ghana in particular is presented. The historical evolution of smoking ovens in those contexts and the food safety hazards associated with the products are also provided.

The experimental work began with a baseline assessment of the food safety status of smoked fish products on markets in Ghana, by screening them for microbiological contaminants, biogenic amines and PAHs (**Chapter 2**). *Salmonella* sp. (the pathogen of interest) was not detected in any of the products (n=80 samples). Histamine (main biogenic amine of interest) and benzo(a)pyrene (PAH marker) both recorded 100% prevalence at concentrations ranging from <10 to 48 ± 7 mg/kg and 11 ± 2 to 75 ±19 µg/kg, respectively. The highest histamine level was four-times lower than the EU maximum limit (200 mg/kg), whereas the benzo(a)pyrene levels exceeded the EU maximum limit (2 µg/kg) by up to 38-times. Using a hazard screening decision flowchart and a proof of concept scoring matrix, PAHs were identified as the most important processing-technique-related food safety hazards in traditionally smoked fish in Ghana.

Since the FTT was developed and introduced mainly to address the problem of high PAHs in smoked fish, its efficacy in that regard was evaluated and confirmed in **Chapter 3**. The mean BaP and PAH4 levels in smoked fish from the FTT were up to 1.8 ± 1.0 µg/kg and 8 ± 3 µg/kg, respectively. The Chorkor smoker products had up to 61 ± 6 µg/kg for BaP

and 395 ± 17 µg/kg for PAH4. For the metal drum, the values were to 70 ± 4 µg/kg for BaP and to 327 ± 37 µg/kg for PAH4. Thus, whereas PAH levels in FTT products were below EU regulatory limits (2 µg/kg for BaP and 12 µg/kg for PAH4), levels in products of the traditional ovens exceeded these limits by up to 33 times. These show that the FTT is efficacious in reducing PAH contamination of fish during smoking.

In **Chapter 4**, a risk assessment was conducted for PAHs in smoked fish from the FTT and the traditional ovens using the margin of exposure (MoE) approach. Whereas the MoE values for FTT products were above 10,000 (as high as 400,000; signifying low public health concern), values for the Chorkor smoker and the metal drum oven were below 10,000 (as low as 1,200; indicating public health concern). Therefore, use of the traditional ovens exposes consumers to PAH health risks, whereas use of the FTT could reduce such exposure to acceptable levels.

Although the FTT was demonstrated to reduce both the occurrence of PAHs and the associated consumer exposure to the hazard in Chapters 3 and 4, respectively, without consumer acceptance of its products, its adoptability will be in question. Therefore, a sensory evaluation was conducted and discussed in **Chapter 5**. No significant differences were found between the consumer liking scores for the sensory attributes of products from the FTT and the Chorkor smoker (the metal drum oven was not tested; it is used much less than the Chorkor smoker in Ghana).

Considering that fish is a rich source of unsaturated fatty acids and that such lipids are susceptible to oxidation, the impact of the oven types and traditional smoked fish storage practices on the stability of lipids in the products was evaluated in **Chapter 6**. It was found that unsaturated lipids in the FTT products were more prone to oxidative degradation than in the Chorkor smoker and the metal drum oven products. It was considered that the higher smoke deposition (observed but not empirically measured) on the products of the traditional ovens provided an anti-oxidative effect through potentially higher levels of phenolic compounds.

In **Chapter 7**, a multicriteria decision analysis (MCDA) was applied to select an intervention for reducing consumer exposure to PAHs in smoked fish in Ghana. The FTT, the Chorkor smoker and a food preparation practice of removing the skin of smoked fish were evaluated as intervention options. The decision criteria were the cost of implementation, food safety, occupational exposure to processing hazards, environmental sustainability, consumer acceptance and sustained use of the proposed intervention. The FTT emerged as the effective intervention, albeit its implementation would require a significant upfront investment.

Finally, in **Chapter 8**, a general discussion is presented with conclusions and recommendations for future research.

SAMENVATTING

Gerookte vis is een belangrijke bron aan dierlijke eiwitten in Ghana. De vis wordt gerookt via typische traditionele ovens ('Chorkor smoker' en metalen vaten), wat resulteert in hoge gehaltes aan polycyclische koolwaterstoffen. Gezien het carcinogene karakter van deze procescontaminanten stelt zich hierdoor een probleem van voedselveiligheid. Een nieuwe oven, de FAO-Thiaroye Technique (FTT), werd ontwikkeld om dit probleem aan te pakken. In deze studie wordt de impact op voedselveiligheid en de humane gezondheid onderzocht door de transitie van traditionele ovens naar de FTT-oven voor het roken van vis in Ghana.

In **hoofdstuk 1**, wordt op basis van een literatuurstudie het belang van gerookte vis als voedsel en bron aan nutriënten in Afrika en Ghana verduidelijkt. De historische evolutie van rookovens en de potentiële gevaren mbt. voedselveiligheid worden hierbij ook belicht. Het experimenteel werk van dit doctoraat startte met een onderzoek van de huidige voedselveiligheidsstatus van gerookte vis aanwezig op de markten in Ghana, met een screening naar microbiologische parameters, biogene amines en polyaromatische koolwaterstoffen (PAKs) (**hoofdstuk 2**). *Salmonella* spp. (als belangrijke pathogeen) werd niet gedetecteerd in de geanalyseerde stalen gerookte vis (n=80). Histamine (belangrijkste biogene amine) en benzo(a)pyreen (BaP, indicator van PAKs) werden in alle stalen teruggevonden boven de detectielimieten, resulterend in een 100% prevalentie. De gemiddelde concentraties varieerden tussen < 10 tot 48 ±7 mg/kg voor histamine en 11 ± 2 tot 75 ±19 µg/kg voor benzo(a)pyreen. De hoogste concentratie aan histamine was 4-maal lager dan de maximale limiet in de EU (M = 200 mg/kg), terwijl de gehaltes aan benzo(a)pyreen de EU limiet (2 µg/kg) tot 38-maal overschrijden. Gebruik makend van een stroomdiagram om gevaren te screenen en een uitgewerkte methode om gevaren te rangschikken, werd besloten dat de PAKs het belangrijkste voedselveiligheidsgevaar zijn voor gerookte visproducten in Ghana.

Aangezien de FTT werd ontwikkeld en geïntroduceerd om de gehaltes aan PAKs in gerookte vis te verlagen, werd de efficiëntie van de FTT geëvalueerd en bevestigd in **hoofdstuk 3**. De gemiddelde BaP en PAH4 concentraties in vis gerookt in de FTT-oven,

zijn respectievelijk, 1,8 ± 1.0 µg/kg and 8 ± 3 µg/kg. De producten gerookt in de Chorkor-oven hadden een concentratie van 61 ± 6 µg/kg voor BaP en 395 ± 17 µg/kg voor PAH4. Producten gerookt door gebruik te maken van de metalen vaten, hadden gemiddelde concentraties van 70 ± 4 µg/kg voor BaP en 327 ± 37 µg/kg voor PAH4. Er kan besloten worden dat de concentraties aan BaP en PAH4 beneden de EU maximale limieten liggen (2 µg/kg voor BaP en 12 µg/kg voor PAH4) voor de gerookte producten bereid met de FTT-oven. De producten, die met de traditionele ovens werden gerookt, overschreden tot 33 keer deze limieten.

In **hoofdstuk 4** werd vervolgens een risico-inschatting uitgevoerd om de impact van de reductie aan PAKs in de gerookte vis te kunnen inschatten. Er werd gebruik gemaakt van de blootstellingsmarge benadering (Margin of Exposure (MoE)). De blootstellingsmarge voor producten gerookt in de FTT-oven waren hoger dan 10 000 (zelfs tot 400 000), duidend op een lage relevantie voor de gezondheidsimpact. Blootstellingsmarges voor de producten gerookt met de Chorkor-oven en de metalen vaten waren lager van 10 000 (zelfs tot 1 200, duidend op een mogelijks probleem inzake volksgezondheid). Er kon besloten worden dat de traditionele ovens de Ghanese bevolking blootstelt aan gehaltes van PAKs die schadelijk zijn voor hun gezondheid terwijl de FTT-oven deze blootstelling sterk reduceert.

Vervolgens werd in **hoofdstuk 5** een consumentenbevraging en sensorische evaluatie uitgevoerd. Het is immers belangrijk dat bij de introductie van een nieuw rookproces de sensorische aanvaarding van de producten ook geëvalueerd wordt. Er werden geen significante verschillen gevonden in de sensorische eigenschappen tussen de producten gerookt met de FTT-oven en de Chorkor-oven (producten gerookt met de metalen vaten werden niet mee opgenomen in deze sensorische evaluatie). Hieruit werd besloten dat de Ghanese consumenten de vis, gerookt via de FTT-oven, ook aanvaarden qua sensorische kwaliteit (oa. uitzicht, smaak, textuur).

Aangezien vis een belangrijke bron is aan onverzadigde vetzuren en deze vetten gevoelig zijn voor oxidatie, werd de impact van de oventypes en de traditionele bewaring van gerookte vis, op de stabiliteit van de vetten in gerookte producten nagegaan **in hoofdstuk 6**. Er werd gevonden dat de onverzadigde vetzuren in de FTT gerookte producten meer onderhevig zijn aan oxidatie tijdens de bewaring, in vergelijking met producten gerookt met de Chorkor-oven en de metalen vaten. De sterkere blootstelling aan rook tijdens het rookproces met de Chorkor-oven en de metalen vaten (geobserveerd maar niet empirisch gemeten) resulteert vermoedelijk in een anti-oxidatief effect door de aanwezigheid van hogere concentraties aan fenolische componenten.

In **hoofdstuk 7**, werd een multicriteria-beslissingsanalyse toegepast om de interventie te selecteren teneinde de inname van PAK’s via de consumptie van gerookte vis te verminderen. De FTT-oven, de Chorkor-oven en het verwijderen van de huid van gerookte vis werden als drie verschillende interventiescenario’s naast elkaar geëvalueerd. De andere criteria die mee in rekening gebracht werden, zijn investeringskost, arbeidsblootstelling aan rook, consumentenacceptatie, duurzaam gebruik van de techniek alsook de milieu-impact. De introductie van de FTT-oven als een technische interventie scoorde het best ten opzichte van de andere twee interventies, maar zal een grote en belangrijke investering vergen bij de implementatie ervan.

Hoofdstuk 8 sluit dit doctoraat af, met een algemene discussie, en aanbevelingen voor verder onderzoek.

GENERAL INTRODUCTION AND RESEARCH OUTLINE

BACKGROUND

Fish is the most important animal protein in Ghana. It is enjoyed in all regions of the country by people of all ages and socioeconomic backgrounds, with upwards of 22% of household food expenditure being made on fish (Atta-Mills et al., 2004; FAO, 2016). Ghanaians, therefore, stand to gain from the nutritional benefits of fish intake, such as its rich supply of essential amino acids, vitamins, minerals and omega-3 polyunsaturated fatty acids (PUFA) which, to various degrees, help to reduce the risk of coronary heart diseases and strokes, prevent mild hypertension and certain cardiac arrhythmias, support neurological development of foetuses (Kris-Etherton et al., 2002) and reduce the risk of cognitive decline in Alzheimer's Disease (Fotuhi et al., 2009).

Realizing the aforementioned gains, however, depends on the safety of the products. Unsafe fish could either rob consumers entirely of the benefits if its consumption results in mortality, or significantly reduce the net gains if it results in, or contributes to, morbidity. Among other factors (such as raw material quality), the safety of food is intrinsically linked to the processing method by which it is produced and the handling practices along the entire value chain. Therefore, the methods of fish processing and handling in Ghana would determine how safe the products are, and what implications this will have for public health.

More than 70% of fish landed in Ghana is processed by traditional methods such as smoking, salting, frying, fermenting, or sun-drying (Nketsia- Tabiri and Sefa-Dedeh, 2000; Adeye and Oyewole, 2016). Among those, smoking is the preferred and most practiced option and is estimated to provide about 80% of the processed form in which the commodity is consumed (Lu et al., 1991; Nerquaye-Tettteh, 2002; Asiedu et al., 2018). Smoked fish, therefore, makes up a significant part of the Ghanaian diet.

The traditional method of fish smoking relies on the use of techniques and tools that compromise the safety of the products. Smoking ovens used are considered rudimentary, sanitary conditions of processing environments poor, and handling during and post-processing unsatisfactory (Brownell, 1983; Sefa-Dedeh 1993; Nketsia- Tabiri and Sefa-

Dedeh, 2000; Asiedu et al., 2018). While poor handling conditions could compromise the microbiological quality and safety of the products, the techniques of smoking could introduce chemical food safety hazards in the products.

Two main types of smoking ovens are used in Ghana. These are the Chorkor smoker and the metal drum oven (further details in Chapter 1). Both have a common operational principle: fish is cooked over dry heat produced by burning fuelwood, and in the process, the product gets flavoured by smoke emanating from the heat source (Brownell, 1983; Nerquaye-Tetteh et al., 2002; Bomfeh et al., 2019). This common principle also confers a common food safety challenge: contamination with polycyclic aromatic hydrocarbons (PAHs). PAHs are organic compounds with genotoxic and mutagenic potential produced when organic materials (such as fuelwood) are exposed to high temperatures and/or pressures (Gehle, 2009; Ciecierska and Obiedzinski, 2007; Mičulis et al. 2011). During the use of traditional ovens, the smoke and direct heat from the fuelwood result in contamination of the products with several PAHs (Stołyhwo and Sikorski, 2005).

In 2014, the Food and Agriculture Organization of the United Nations (FAO) introduced an improved oven in Ghana with the view to improving the safety of smoked fish. The oven, called the FAO-Thiaroye Processing Technique (FTT), was developed mainly to reduce PAH contamination during fish smoking (Ndiaye and Diei-Ouadi, 2011). The efficacy of the innovation had, however, not been scientifically established. This study was therefore conducted *to primarily investigate the extent to which the use of the FTT could reduce PAH contamination of fish during smoking, and to determine the effect of such reduction, if any, on public health*. In addition, the study investigated the occurrence of other food safety hazards, consumer acceptance of smoked fish products from the FTT and other factors related to food safety governance, as set forth next.

SPECIFIC OBJECTIVES

The specific objectives of the study were:

1. To screen smoked *Sardinella* sp. and *Sphyraena* sp. sourced from selected markets and a fish processing site in Ghana for polycyclic aromatic hydrocarbons

(PAHs), biogenic amines and microbial quality and safety contaminants; and to determine the most relevant hazard for public health vis-à-vis the processing technique.

2. To determine the impact of the FTT on PAH contamination levels in fish during smoking, through comparative smoking experiments with the traditional ovens (Chorkor smoker and the metal drum oven)
3. To evaluate consumer exposure to PAHs in smoked fish produced with the FTT versus the traditional ovens by probabilistic exposure assessments
4. To assess consumer acceptance of smoked fish from the FTT
5. To qualitatively explore the occurrence of epoxy fatty acid species in smoked fish in order to evaluate their potential use as oxidation markers in smoked fish lipids
6. To conduct a multi-criteria decision analysis to select a suitable intervention option for reducing consumer exposure to PAHs in smoked fish in Ghana

ORGANISATION OF THE THESIS

This thesis is organised into eight (8) chapters, as summarised in the schematic overview (Fig.01). **Chapter 1** presents a review of the literature on traditional fish smoking methods in Africa with emphasis on Ghana, the contribution of smoked fish to food and nutrition security, livelihood support and economic development in that context, and the nature, occurrence, health impacts and risk management strategies of selected food safety hazards associated with the products. In **Chapter 2**, the prevailing situation of smoked fish safety in Ghana is evaluated through a testing of the products from informal markets and processing sites for three main food safety hazards. The identified hazards are then screened in terms of their relevance for food safety governance within the Ghanaian context. The efficacy of the FTT to reduce PAH contamination of fish during smoking is discussed in **Chapter 3**, along with an assessment of the effect of the operational and design features of the innovation on PAH contamination. This is followed by a risk assessment in **Chapter 4,** determining the public health burden of PAHs individually linked with the use of the FTT and the traditional ovens. **Chapter 5** presents a sensory evaluation of smoked fish produced with the FTT and a traditional oven, ascertaining if consumers could notice a difference between, and state a preference for, products from the two ovens. In **Chapter 6**, an exploratory qualitative characterisation of epoxy fatty acids in smoked fish lipids is presented. **Chapter 7** details a multicriteria decision analysis for the selection of an intervention for reducing PAH exposure in smoked fish in Ghana, considering several factors beyond public health impact. Finally, a general discussion of the study findings with conclusions and recommendations for future (research) work is given in **Chapter 8.**

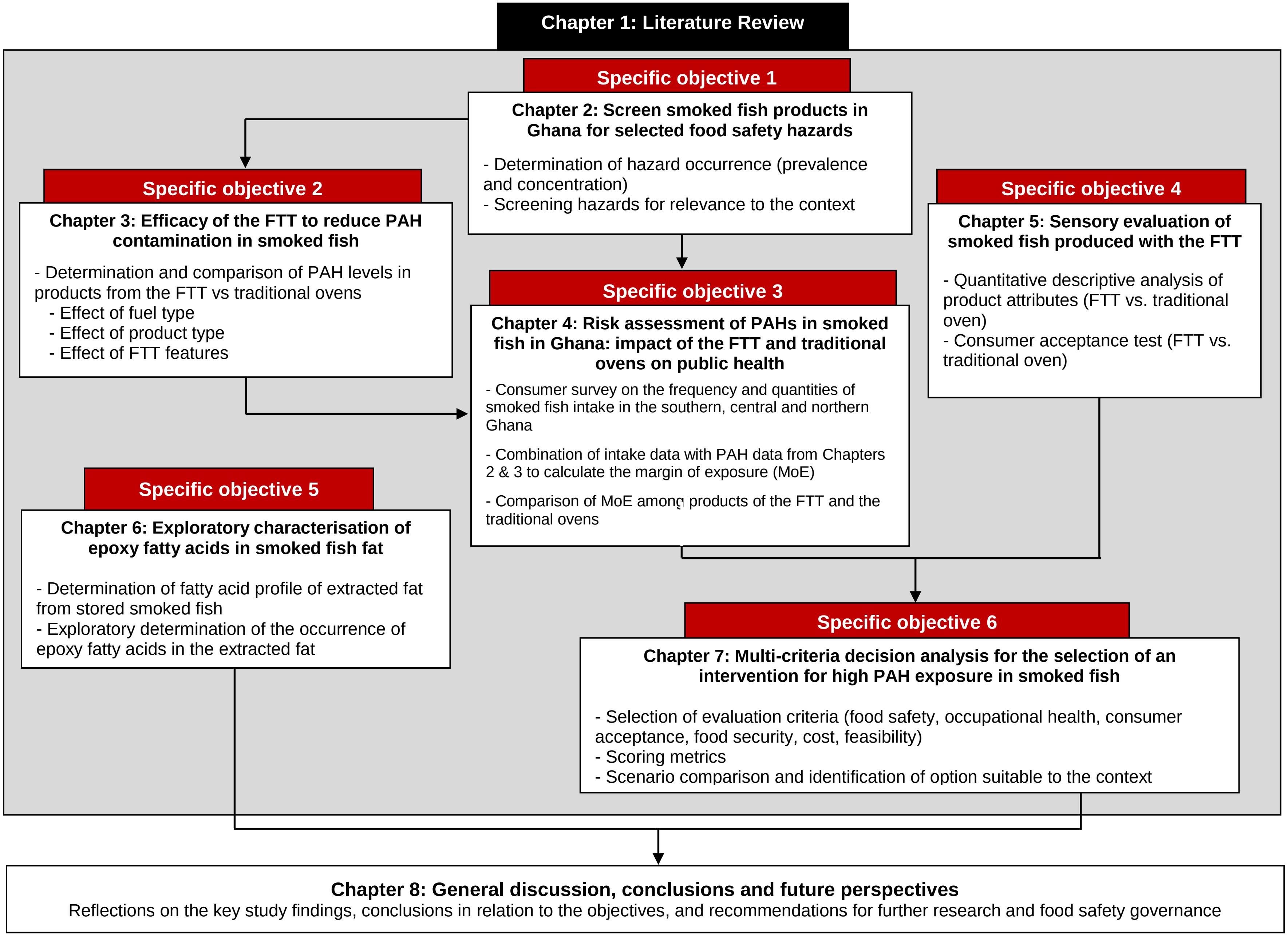


Fig. 01: Schematic overview of the thesis

CHAPTER 1: LITERATURE REVIEW

1.1 The Ghana fishery sector

The fishery sector in Ghana is a major contributor to revenue generation and food security for the country. At the international front, they account for over 50% of the country's earnings from non-traditional exports (NTE) (GEPC, 2010). The products are considered Ghana's second most important NTE after horticultural commodities (BoG, 2008), adding an estimated USD 300 million annually to export revenue (Baidoo-Tsibu, 2016). Altogether, the sector contributes over 5% of the country's Gross Domestic Product (GDP) and 12% of the agricultural GDP (GEPC, 2010; FAO, 2016).

At the domestic front, the sector provides full-time and seasonal employment for many citizens who work at various points along the fish value chain. It is estimated that more than 10% of the country's population obtain their livelihoods through direct involvement in the sector (Overa, 2002; Atta-Mills *et al*., 2004). Women are the key players in the post-harvest arm of the value chain, while men are primarily involved in fishing (Aduomih, 2019).

In terms of food security, the sector supplies the major source of animal protein for the country. With an average per capita intake of 27 kg – close to three times the estimated Africa average of 9.7 kg – fish makes up about 60% of animal protein in Ghanaian diets (FAO, 2016). Recent estimates place Ghana's per capita intake between 20 - 30 kg and that for Africa at 9.9 kg (SOFIA, 2020). Globally, the contribution of fish to animal protein in diets is 18% (FAO, 2017). Fish also accounts for 22.4% of total food expenditure in households in the general population and 25.7% in low-income households (Atta-Mills *et al*., 2004; Ghana Living Standards Survey, 2008; FAO, 2016).

The country has three fisheries sub-sectors – marine, inland and aquaculture – each respectively accounting for 70%, 17% and 13% of total fish production (MoFAD, 2017). Whereas the country's annual fresh fish requirement is 720 000 metric tonnes, supply stands at 400 000 metric tonnes, making the nation a net fish importer (FAO, 2016; MoFAD, 2017).

1.2 Significance of traditional fish smoking in the Ghanaian fishery sector

The bulk of fish consumed in the country is traditionally processed by smoking, sun-drying, deep frying, salting and fermenting (Nketsia-Tabiri and Sefa-Dedeh, 2000; Adu-Gyamfi, 2006). Of these methods, traditional smoking is the most practiced. Smoked fish remains the preferred product of all locally processed fish in Ghana (Lu et al., 1991; Adu-Gyamfi, 2006).

The reasons and benefits noted for the preference of fish smoking in Ghana are that it makes fish available all year round, imparts a smoky flavour to the products, increases fish utilization in traditional meals, reduces waste during bumper harvests, and facilitates the packaging, transportation and marketing of the commodity (Nerquaye-Tetteh, 1999).

Fish smoking is also a major driver of economic activities in coastal and riparian communities, providing livelihood support to several thousands (SNV, 2016). While those involved in processing and bulk-trading the product obtain direct employment, others such as oven builders, fuelwood suppliers, basket weavers (making containers for the smoked fish) and opportunistic retailers also obtain ancillary employment (FAO, 2016; Mindjimba et al., 2019). It has been estimated that twice as many dependents as actors are supported by earnings from the value chain (Asafo, 1995).

From the foregoing, it is seen that smoked fish production and consumption significantly bolster Ghana's efforts at pursuing the Sustainable Development Goals (SDGs) of the United Nations (UN, 2015) through provision of employment (SDG1), reduction of hunger (SDG 2), provision of fish protein for good health and wellbeing (SDG 3), support for the economy (SDG 8), and prevention of postharvest losses through processing and efficient utilization of fishery resources (SDG 12).

1.3 Traditional fish smoking practices in Ghana

In general, there are two types of smoking processes for food. These are cold smoking and hot smoking (CAC, 2013). Cold smoking is practiced in advanced countries and typically aims to impart a smoky flavour to food without cooking it. It is carried out at temperatures between 20-30°C (Cardinal et al., 2001). In hot smoking, temperatures of 50-80°C are used to cook and flavour the product (Bilgin et al., 2008).

Traditional fish smoking in Ghana is a hot smoking process. It involves simultaneous cooking and flavouring of fish over dry heat and smoke from fuelwood on traditional ovens (Nerquaye-Tetteh, 1999). The cooking temperatures range from 80°C to 100°C with processing times ranging from three hours for a moist product (called smoked-soft fish, moisture content ≥20%), or ≥10 hours for a dry product (smoked-dry fish, moisture content ≤20%) that can keep for up to nine months at ambient temperature (Nerquaye-Tetteh, 1999; SNV, 2016).

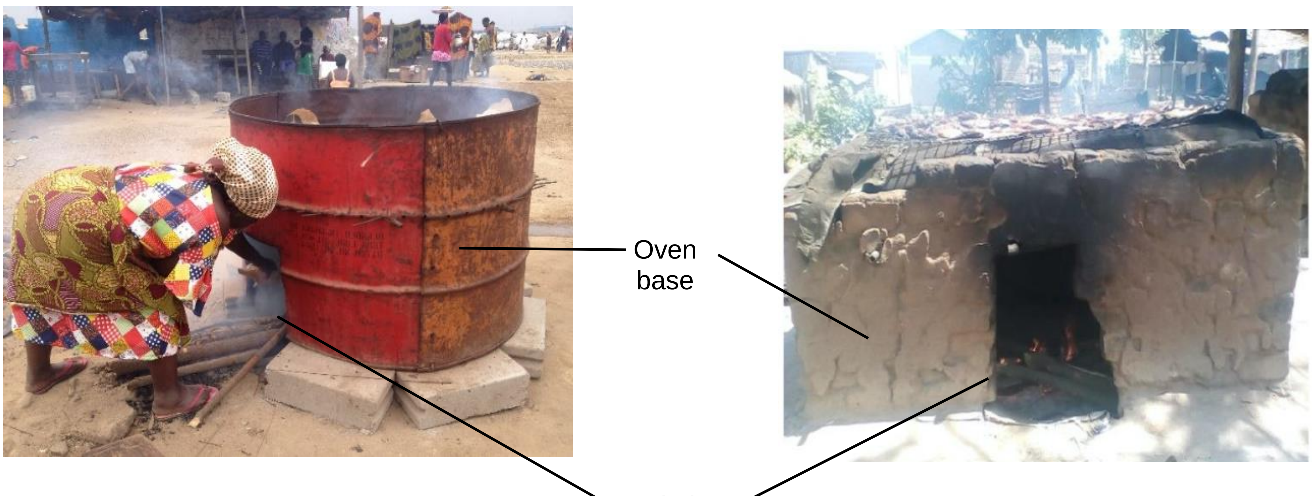
1.3.1 Ovens for traditional fish smoking

Historically, smoking ovens in Ghana have differed in shape (cylindrical vs. rectangular) and construction material (metal vs. mud). Prior to the 1960s, four main ovens were identified: cylindrical mud ovens (also called "Fante oven[1]"), cylindrical metal oven (also called "metal drum oven"), rectangular mud oven, and rectangular metal oven (Nerquaye-Tetteh, 1999). Regardless of shape or construction material, these ovens consisted of a base having a stokehole for fuelwood (Fig.1.1). Sticks or metal rods were suspended above the stokehole to hold fish during smoking. A maximum of three layers of fish (approximately 20kg), separated by thin sticks, could be processed at a time. The metal drum was the most widely used among the four (Nti et al., 1999).

Although these ovens required little upfront investment, they had several challenges such as low capacity, low fuel efficiency, excessive handling of fish during smoking, uneven quality of smoked products and excessive exposure of processors to wood smoke (Brownell, 1983). Their low capacity contributed to high post-harvest losses during bumper seasons (Peñarubia et al., 2017). The challenges resulted in several efforts in Ghana and other African countries to develop improved ovens, mainly focusing on increasing throughput and fuel efficiency, reducing drudgery and enhancing organoleptic

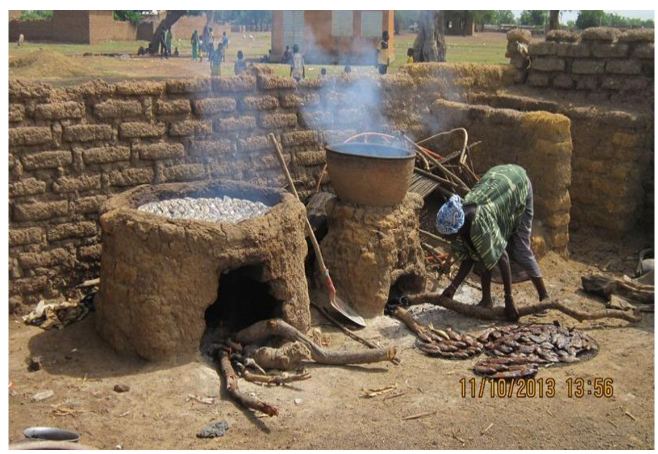
[1] This oven derived its name from its widespread use among the Fante ethnic group in the Western and Central Regions of Ghana (Nerquaye-Tetteh, 1999)

properties of smoked products (Nerquaye-Tetteh, 1999). This led to the introduction of the first-generation of improved ovens.



a) Cylindrical metal oven (metal drum oven) **b) Rectangular mud oven**

c) Fish arranged on the metal drum oven



d) Cylindrical mud oven (Fante oven, left), and a traditional mud oven (dafing) in Burkina Faso (right)
[Source: Mindjimba et al., 2019]

Fig. 1.1: Traditional fish smoking ovens in Ghana

1.3.2 First-generation of improved ovens

Several improved ovens were developed and tested. These included the Adjetey, the Ivory Coast, the Banda, and the Altona ovens (Nerquaye-Tetteh, 1999), each having peculiar disadvantages. The Adjetey and Ivory Coast ovens, for example, were considered expensive, cumbersome to use and unable to yield better quality fish than the traditional ovens. The Altona was considered costly and culturally unacceptable. Its use required fish to be skewed through the eyes and suspended above the heat source. That practice was alien to processors; they were used to arranging fish flat on a grill and could not accept hanging their products during processing (Peñarubia et al., 2017). Consumers, on the other hand, were unaccustomed to purchasing fish that had no eyes (Nerquaye-Tetteh, 1999).

In 1969, the Food and Agriculture Organization of the United Nations (FAO), in partnership with the Food Research Institute (FRI) of the Ghana Centre for Scientific and Industrial Research (CSIR), introduced an oven called the Chorkor smoker. This was developed with the active involvement of women fish processors in Chorkor, a peri-urban fishing community in Accra (Ghana's capital). The oven derives its name from the community (Brownell, 1983; Nerquaye-Tetteh et al., 2002). Its development was heavily based on traditional processing practices to avoid a marked departure from the preferences of the end users.

Like the traditional ovens, the Chorkor smoker had a base for holding the fish and stokeholes for fuel (Fig. 1.2). Unlike the traditional ovens, it had two compartments, two stokeholes and a stack of racks for holding fish during smoking, thereby significantly increasing the volume of fish that could be smoked at a time (capacity of up to 15 racks, each holding about 20kg) (Brownell, 1983; Nerquaye-Tetteh, 1999). During use, the racks are intermittently reshuffled to allow uniform cooking of the fish.

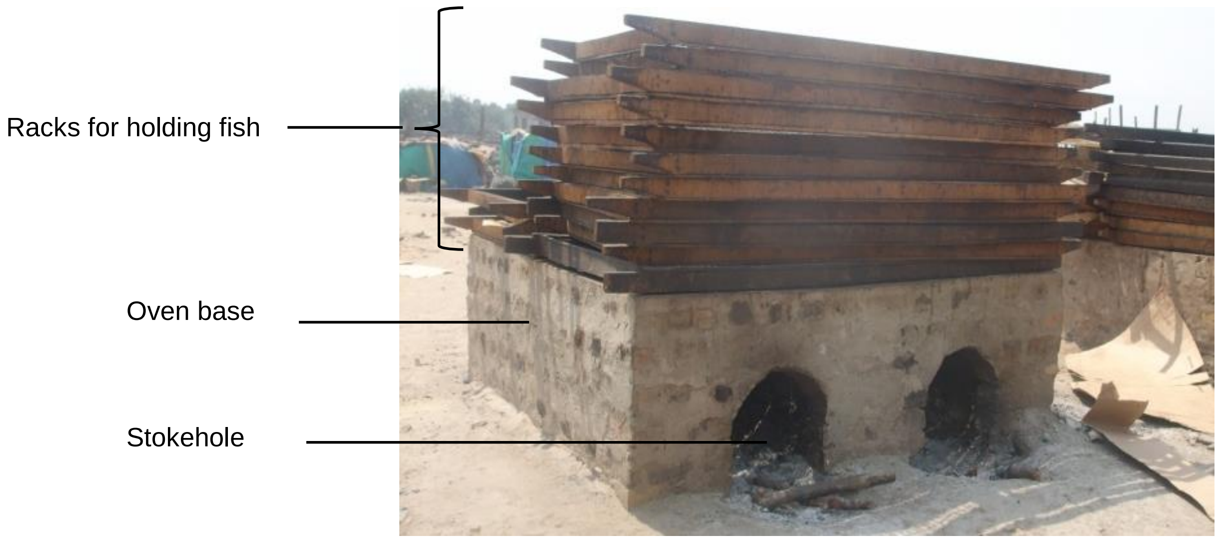


Fig. 1.2: The Chorkor Smoker

Due to its advantages of higher capacity, higher fuel efficiency and reduced occupational exposure to smoke and burn injuries, this innovation adequately addressed the known shortcomings of the traditional ovens and was widely accepted in Ghana (Nti et al., 1999). It was subsequently adopted in several African countries. However, a few countries, such as The Gambia, Sierra Leone and Senegal, were slow in adopting it due to the labour demand for reshuffling the racks (Peñarubia et al., 2017). As a result, modifications of the Chorkor smoker were made in such countries to suit their preferences while maintaining the focus on throughput improvement, drudgery reduction and sensory appeal enhancement (Peñarubia et al., 2017).

The introduction of the Chorkor smoker did not automatically phase out the metal drum oven. To date, although limited, it is still used in Ghana and elsewhere, especially when small quantities of fish are smoked, or during lean seasons. The Chorkor smoker, however, continues to be the preferred first-generation improved oven for fish smoking in Ghana in particular and in Africa in general. Its advantages notwithstanding, the potential for introducing food safety hazards from poor handling practices and the use of fuelwood remain.

1.3.3 Second-generation of improved ovens: from higher yield to safer fish

In the mid-2000s, international market and regulatory forces compelled a shift in oven innovation focus in Africa from higher yield to safer products (FAO, 2007). This was due to border rejections of African smoked fish in the European Union (EU) on account of their high levels of polycyclic aromatic hydrocarbons (PAHs). (Exports of smoked fish from Africa are targeted at the diaspora (Asiedu et al., 2018)). At the time, the maximum limit of the PAH marker (benzo(a)pyrene, BaP) was 5µg/kg (Commission Regulation (EC) No. 1881/2006). In response, several attempts were made by research institutions on the continent to develop a second-generation of improved ovens that could lower the contaminant levels below the EU limit. In Gabon, for example, the *Institut de Recherche Technologique (IRT)* (Technological Research Institute) developed an oven with an indirect smoking system; it failed to meet the limit (Peñarubia et al., 2017). While efforts continued, even stricter regulations for PAHs were introduced, with a reduction in the maximum limit of BaP from 5µg/kg to 2µg/kg (Commission Regulation (EC) No. 835/2011).

From 2009, with the support of the FAO, the Senegal *Centre National de Formation des Techniciens des Pêches et Aquaculture* (CNFTPA) (National Training Centre for Fisheries and Aquaculture Technicians) began a series of oven development trials that ended in a prototype which, upon preliminary testing, gave PAH results lower than the EU limit (Diei-Ouadi and Ndiaye, 2011; Peñarubia et al., 2017). The innovation was called the FAO-Thiaroye Processing Technique (FTT), named after Thiaroye, the historic Senegalese town (FAO, 2014). It was designed to add food safety control to the established benefits of the first-generation improved ovens.

Beyond food safety, the FTT was envisaged to offer the following benefits (Ndiaye et al., 2015):

i. *Higher income for processors*: Fish smoked with FTT would have added value (safer) and gain access to more rewarding markets
ii. *Ancillary employment*: Local artisans involved in the construction and installation of the oven could obtain opportunistic employment or additional business

iii. *Mainstreaming gender concerns*: By reducing occupational exposure to smoke, the health of processors (women) would be protected.

iv. *Environmental protection*: It favours reduced deforestation due to its fuel efficiency and supplementation of same with heat-retention stones. The system is also adaptable to the use of renewable energy (e.g. (bio)gas).

1.3.3.1 Features of the FTT

The FTT consists of an oven base, an ember furnace to hold the cooking fuel, a fat collector to direct fish fat exudates away from the heat source into a reservoir, and a smoke generator (Fig. 1.3 – 1.5). The smoke generator is made up of a chamber within which smoke is produced and a filtering unit to reduce the tar content of the smoke. All these components are made with galvanized steel (Ndiaye et al., 2015).

An innovative construction feature of the FTT is that it can either be built from scratch, or the metallic components could be retrofitted to an existing first-generation improved oven (Fig. 1.6) (Ndiaye et al., 2015; Zelasney et al., 2020).

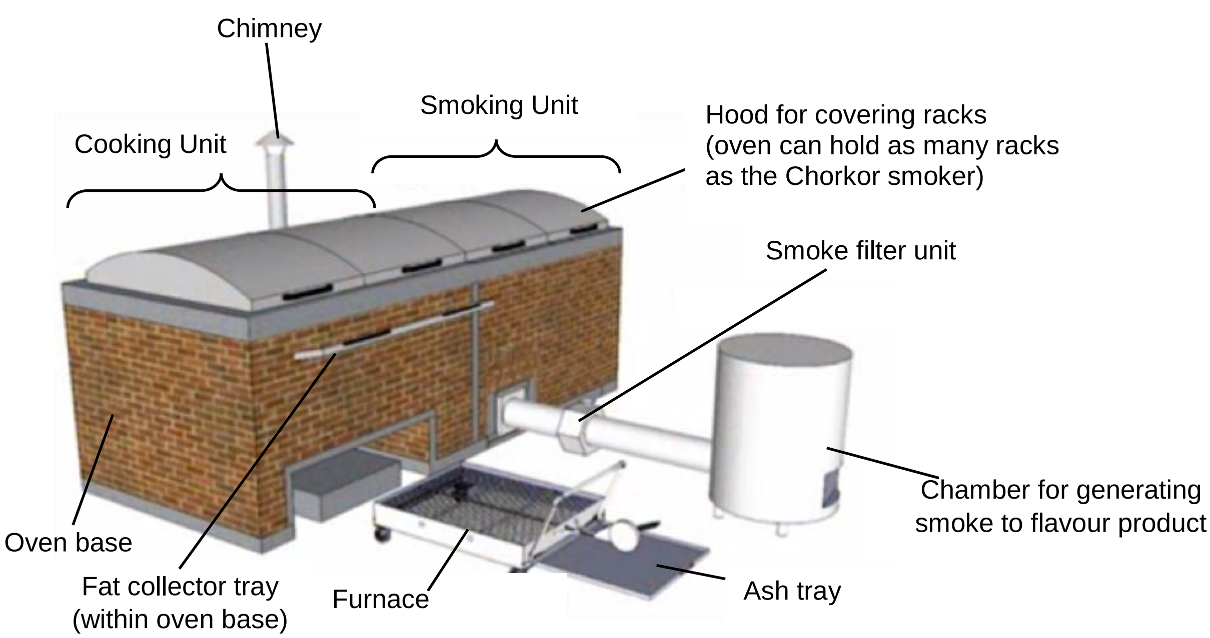


Fig. 1.3: Basic schematic of the FTT (Ndiaye et al., 2015)

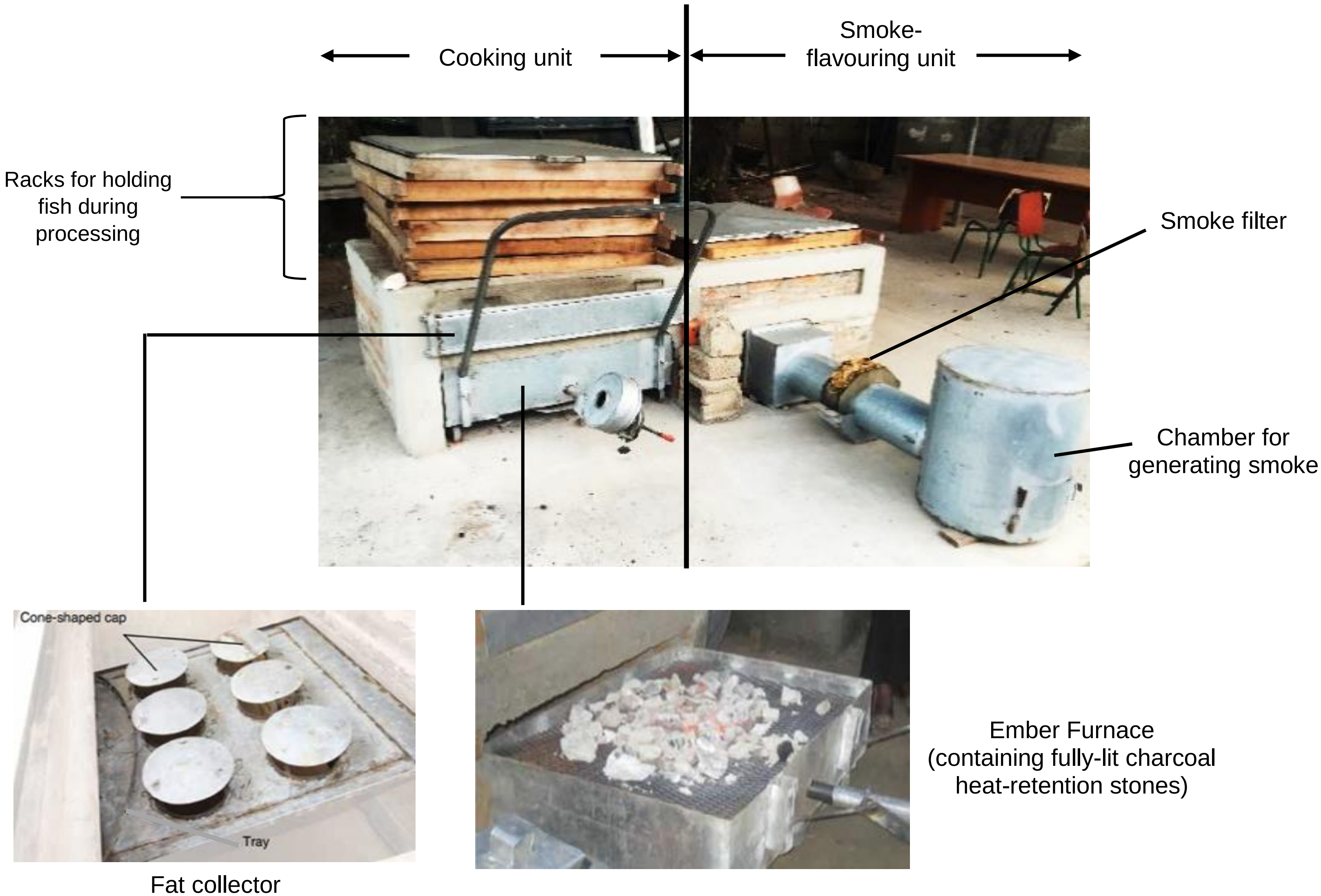


Fig. 1.4 Components of an FTT oven. During processing, pre-cleaned raw fish are arranged on the racks and first cooked over the cooking unit, and then transferred to the adjoining smoke-flavouring unit to be flavoured with filtered smoke. The fat collector drains fat exudates from the fish (at the cooking step) into an external reservoir behind the oven base.

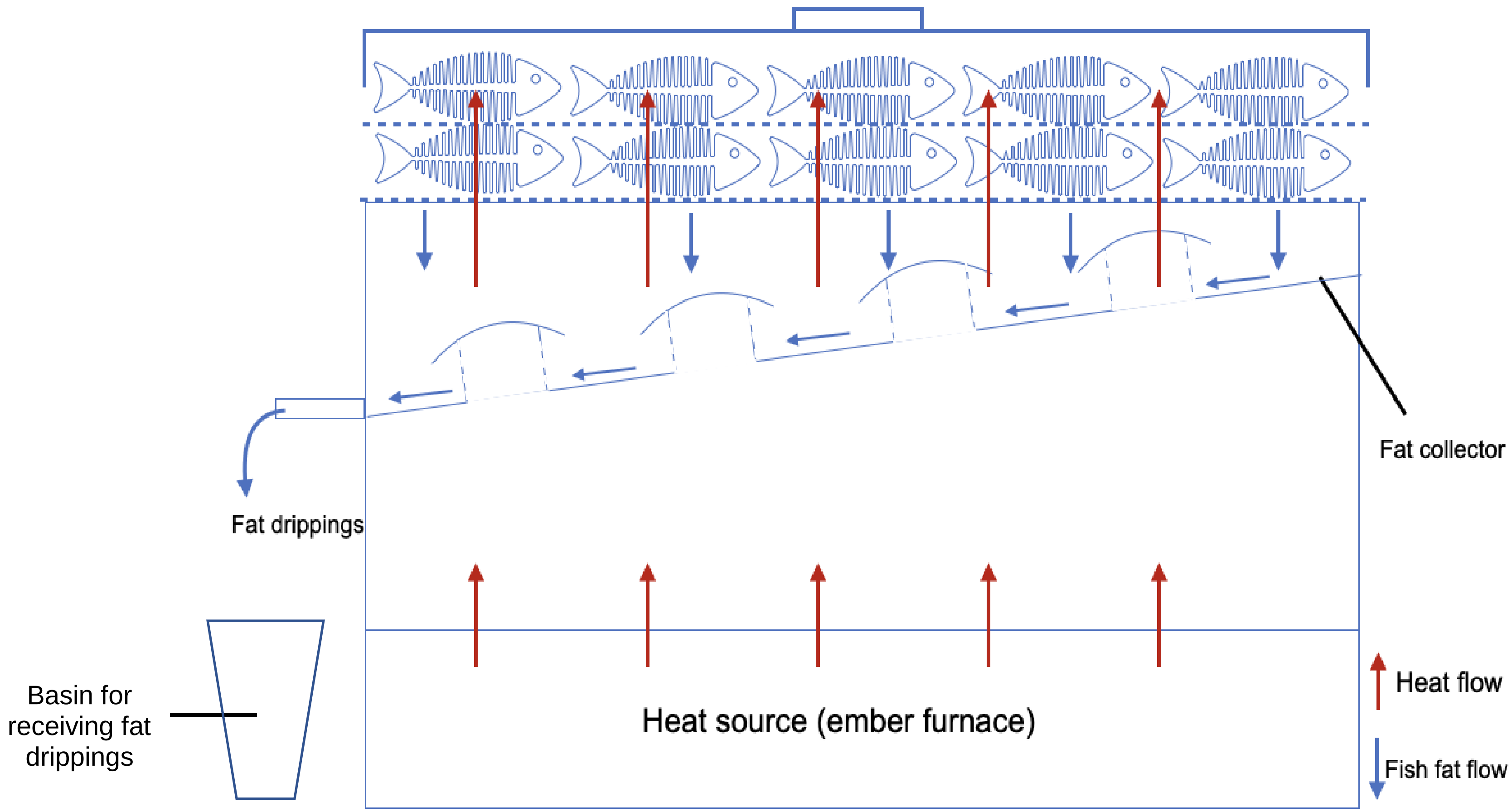


Fig. 1.5: An illustrated transverse section of an FTT oven to show movement of fat drippings from fish during the cooking step

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Chorkor smoker

FTT derived from the Chorkor smoker

Dafing oven (round mud oven used in Burkina Faso)

FTT derived from the dafing oven

The Banda oven

FTT derived from the Banda oven

Fig. 1.6: Traditional or first-generation improved ovens (left) and their corresponding FTT versions. The FTT versions are made by addition of the ember furnace, fat collector and indirect smoke generator. These components can be fitted to the base of an existing first-generation improved oven.

1.3.3.2 Operational differences between traditional ovens and the FTT

As depicted in Figs. 1.7 and 1.8, fish smoking with traditional ovens entails washing fresh (or thawed) fish, eviscerating (optional) and arranging them on the grill of the metal drum oven or on the racks of the Chorkor smoker. They are left to air-dry for about 15 minutes and then subjected to heat and smoke treatment (Oppey, 2002; Bomfeh, 2011). The air-drying enhances the uptake and retention of the smoke flavour (Varlet et al., 2007). Heat energy and smoke transfer to the products is direct and constant throughout the processing, although the intensities of both may be slightly reduced towards the end of the operation.

Fish smoking with the FTT, on the other hand, is divided into two distinct unit operations after the pre-processing activities (washing, evisceration, arranging on trays, air drying):

- the fish is first cooked over dry heat from a combination of fully-lit charcoal and heat-retention materials such as broken clay pottery (Fig. 1.4) (Ndiaye et al., 2015). Cooking temperature ranges from 80-90^{o}C (Diei-Ouadi and Ndiaye, 2011). The cooking duration depends on the desired end product (smoked-soft or smoked-dry).

- At the end of the cooking step, the trays are transferred to the adjoining compartment, where the products are exposed to filtered smoke for approximately 30 minutes (Ndiaye et al., 2015). The smoke is produced from moistened, lit sugarcane bagasse. Moistened luffa sponge is used as filter to trap tar particles to potentially reduce PAH deposits on the product (ibid.). The differences in operational principles and process flow of the FTT and the traditional ovens are summarized in Table 1.0 and Fig. 1.8.

Description of some steps in the traditional smoked fish value chain	Metal drum oven	Chorkor smoker

Traditional ovens: The Chorkor smoker (right) was developed in 1969 to improve the throughput of the metal drum oven (left) and reduce the processing drudgery. The basic operational principles and handling practices are the same.

Fish being washed prior to smoking: Fresh/thawed fish is washed in potable water before smoking. Large fish are cut up before processing, usually into three pieces (head, mid piece and caudal piece)

Fish on smoking racks: For the Chorkor smoker, washed fish are loaded on racks. The Chorkor smoker can take up to 18 racks of fish, whereas the metal drum oven takes about 3 layers separated by sticks

Finished products: Smoked products are sent immediately to the market. Smoked-dry products can however be stored for up to nine months.

Smoked fish displayed for sale in a market in Ghana: Products are subject to ambient temperature and contamination from the environment. There are no cold storage facilities for the product on such markets. This is a common practice for products from both ovens.

Storage of smoked-dry products
Racks of smoked-dry products are stored this way for up to nine months, with occasional re-smoking to control insect infestation.

Fig. 1.7: Pictorial description of some steps in the traditional smoked fish value chain (pictures on the left relate to the use of the metal drum oven; those on the right relate to the Chorkor smoker, and those in the middle are common to both ovens

Table 1.0 Summary of operational differences between the FTT and the traditional ovens

Parameter	FTT	Traditional ovens
Heat source	Fully-lit charcoal combined with heat-retention stones (such as broken pottery)	Fuelwood
Heat transfer	Indirect	Direct
Smoke transfer	Indirect	Direct
Smoke filtering	Filtered	Not filtered
Cooking operation	Separate cooking and smoke flavouring unit operations	Simultaneous cooking and smoke flavouring
Control of fat exudate	Collected and drained away from heat source into an external reservoir	Fat drips directly into heat source

Based on Ndiaye et al., 2015

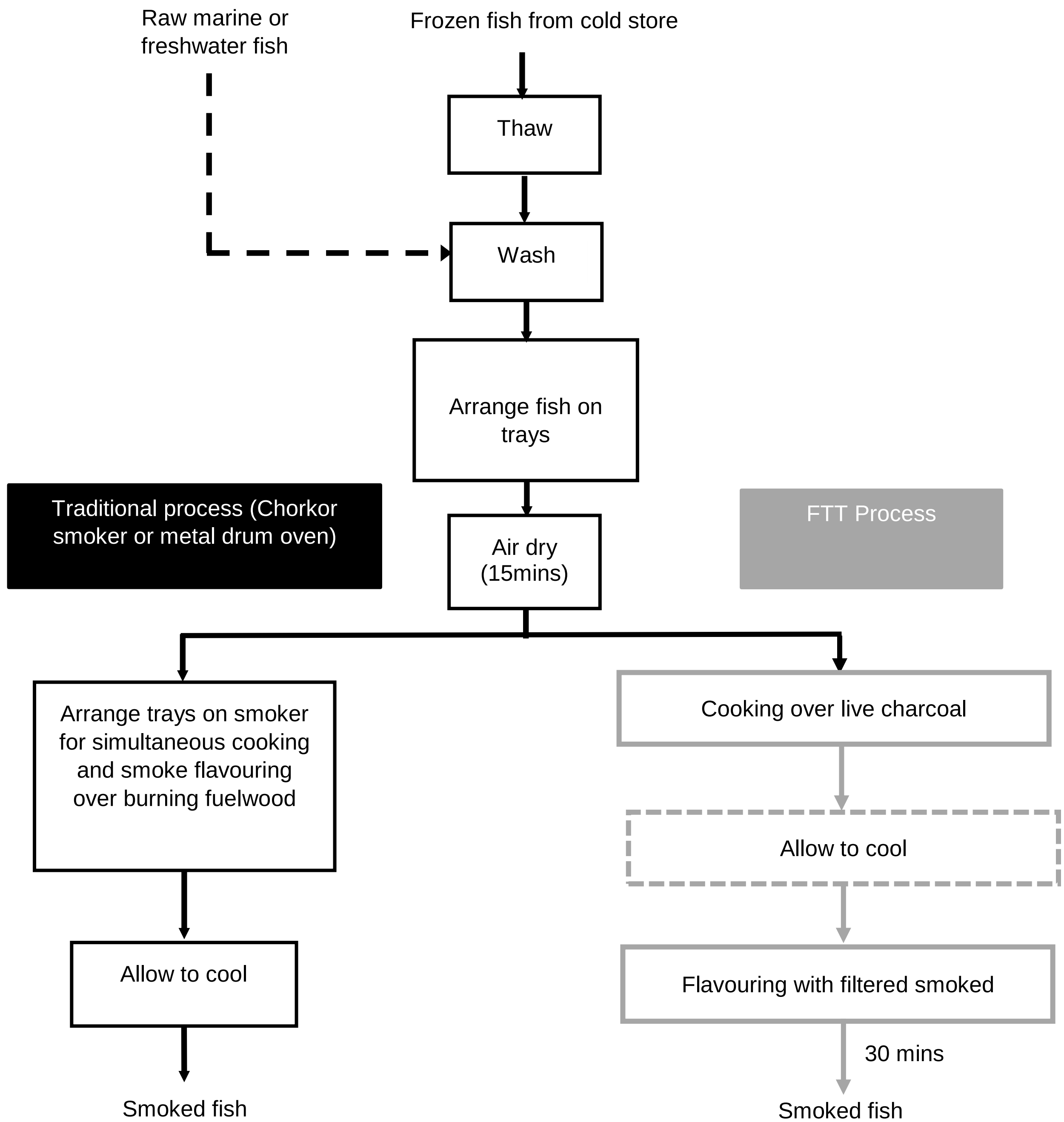


Fig. 1.8: Comparison of the general process flow of fish smoking with traditional ovens and the FTT. (Note: small fish such as anchovies are not washed before smoking)

1.4 Food safety challenges with traditional fish smoking

Over the years, studies have shown that traditional fish smoking practices in Ghana are fraught with food safety challenges, principally due to poor fish handling before, during and after processing, the techniques of smoking, and the unsanitary conditions of markets (Bomfeh, 2011). A brief review on the nature, occurrence and regulation of some hazards linked to the processing and handling of the products are presented next.

1.4.1 Microbiological contaminants in smoked fish in Ghana

Bacteria pathogens associated with fish are categorized into two groups: indigenous and non-indigenous pathogenic flora (Arias, 2009). The indigenous pathogenic flora is commonly found in the aquatic environment and may be present on harvested fish (Huss et al., 2004). Their presence in the final product, however, depends on the lethality of the processing conditions applied. Non-indigenous pathogenic bacteria are not naturally present on fish but are associated with the environment and human or warm-blood animals and their faeces (Uyttendaele et al., 2018). They contaminate fish through poor handling (Arias, 2009). Examples of pathogens in each category are given in Table 1.1.

Although the pathogens potentially associated with fish are diverse (Table 1.1), the actual types detected are often few, due to their poor competition with spoilage bacteria (Arias, 2009). Among the hazards, *Salmonella* sp., *Listeria monocytogenes* and *Vibrio parahaemolyticus* are of particular concern (Uyttendaele et al., 2018). In 2012, *Salmonella* sp. in salmon caused an outbreak with 1,149 cases and four deaths in the Netherlands (Uyttendaele et al., 2018).

In the case of traditionally smoked fish, the heat applied may be sufficient to eliminate several heat-labile pathogens. However, in the absence of good post-processing sanitary control during transport, marketing and consumer handling, recontamination could occur (Uyttendaele et al., 2018).

1.4.1.1 Occurrence of microbial hazards in traditionally smoked fish

The lack of cold storage facilities along the value chain and poor hygienic handling of fish in developing countries predispose products to contamination with several microbiological contaminants (Sefa-Dedeh, 1993; Nketsia-Tabiri and Sefa-Dedeh, 2000; Bomfeh, 2011). Bacteria such as *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus, Micrococcus* sp. and *Proteus* sp. have been isolated in smoked fish on markets in Ghana (Oppey, 2002; Nyamekye, 2000; Adu-Gyamfi 2006).

Table 1.1: Typical indigenous and non-indigenous bacterial pathogens in fresh fish

Indigenous			Non-indigenous		
Organism	**Aquatic temperature for growth**	**Estimated minimum infective dose**	**Organism**	**Primary habitat/Source**	**Minimum infective dose**
Clostridium botulinum (non-proteolytic type E)	3 – 26°C	0.01-1 µg toxin lethal dose	*Listeria monocytogenes*	Soil, birds, sewage, estuarine environments, mud	Variable depending on strand. Generally >10^2 cells/g
Vibrio cholerae *Vibrio parahaemolyticus* Other pathogenic Vibrios spp.	10 – 37°C	10^5 – 10^6 cells/g	*Staphylococcus aureus*	Ubiquitous, human origin	10^5 – 10^6 cells/g Toxin levels 0.14 – 0.19 µg/kg body weight
Aeromonas spp.	5 – 35°C	Unknown	*Salmonella spp.*	Human origin	10^1 – 10^2 cells
Plesiomonas shigelloides	8 – 37°C	Unknown	*Escherichia coli*	Faecal contamination	10^1 – 10^3 cells
			Yersinia enterocolitica	Ubiquitous in environment	10^7 – 10^9 cells/g
			Clostridium botulinum (proteolytic type A, B)	Soil, decaying vegetation, ubiquitous in temperate environment	0.01-1 µg toxin lethal dose
			Clostridium perfringens	Soil (type A), animals (types B, C, D, E)	Unknown. Generally considered >10^6 cells/g
			Bacillus spp.	Ubiquitous, soil, vegetation	10^4-10^9 cells/g

Source: Adapted from Huss et al., 2004; Arias, 2009

1.4.1.2 Regulation of microbiological hazards

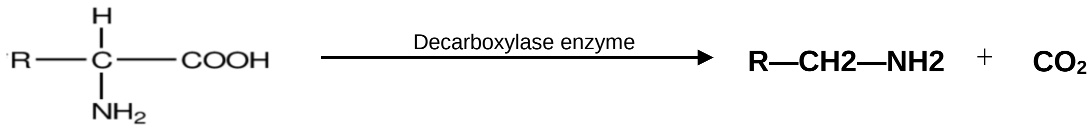
The Codex Alimentarius Commission (CAC, hereafter called Codex) requires all smoked fish products to be prepared under conditions that accord with the General Principles of Food Hygiene (CXC 1-1969) and the Code of Practice for Fish and Fishery Products. The Codex standard for smoked fish, smoke-flavoured fish and smoke-dried fish (CXS 311-2-13) also requires regional and national regulations on microbiological safety of the products to first comply with the Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods (CXG 21-1997, revised in 2013).

In the EU, Commission Regulation (EC) No. 2073/2005 defines the microbiological criteria for foods (EFSA, 2005), with a legal limit of 100 CFU/g for *Listeria monocytogenes* in smoked fishery products at the end of their shelf-life (EFSA, 2005). No limit is defined for *Salmonella* spp. in smoked fish, although in cooked fishery products (refrigerated) the requirement is absence in 25 g. Uyttendaele et al. (2018) recommend the application of the absence in 25 g rule to smoked fishery products for *Salmonella* sp.

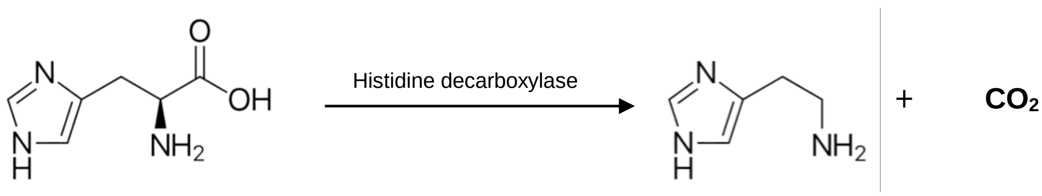
The Ghana Standards Authority introduced two instruments to regulate the microbiological quality of fish in Ghana. These are the Code of Hygienic Practices for Fish (GS 929) and Requirements for Establishing an Own-Checks System (GS 236). The former draws on Codex provisions to define appropriate sanitary practices for fishery products, whiles the latter provides guidance on the establishment of a self-checking food safety management system within processing facilities (GSA, 2018). These, however, are chiefly targeted at export-oriented establishments.

1.4.2 Biogenic amines

Biogenic amines are compounds formed in food by the microbial decarboxylation of the corresponding free amino acids (Silla Santos, 1996; Shallaby, 1997; Park et al., 2010; Zhai et al., 2011). The decarboxylation reaction is illustrated in Fig. 1.9. Fish is a particularly suitable substrate for the formation of these compounds due to its rich amino acid content. The level of biogenic amines in fish is used as a quality index and serves as an important tool for sanitary surveillance (Moreno and Torres, 2001; Vinci and Antonelli, 2002).



a) General amino acid decarboxylation reaction



b) Decarboxylation of histidine to histamine

Fig. 1.9: Formation of biogenic amines by enzymatic decarboxylation [General reaction (a) and specific example of histamine formation from histidine(b)]

The formation of biogenic amines in fresh fish largely depends on post-harvest and pre-processing handling practices, since those practices determine the extent of enzymatic and bacterial action on fish proteins. Freshly caught fish, if not subjected to prompt temperature control, undergoes rapid deterioration. The spoilage generally progresses through rigor mortis, enzymatic autolysis, lipid oxidation and microbial growth (Ghaly et al., 2010). Rigor mortis refers to the loss of fish flexibility due to stiffening of muscles after death. Its effect on fish quality include excessive drip loss post-rigor, and breakdown of connective tissue causing fish muscle to come apart (especially in fillets) (Stroud, 2001). Enzymatic autolysis refers to the breakdown of fish molecules by endogenous enzymes. It results in textural changes and the release of free amino acids, which is an important contributor to the eventual formation of biogenic amines. Lipid oxidation in fish refers to both enzymatic and non-enzymatic hydrolysis of fish fat, resulting in rancidity (Ghaly et al., 2010). Microbial invasion accelerates fish spoilage, producing compounds such as biogenic amines, sulphides, aldehydes and ketones, thereby producing the unpleasant smell of putrefaction (Dalgaard et al., 2006).

Among the aforementioned phases of fish spoilage, the enzymatic autolysis and microbial activity are the most important for the formation of biogenic amines, as these directly contribute to the availability of free amino acids, which are the precursors of the biogenic amines (Fig. 1.10).

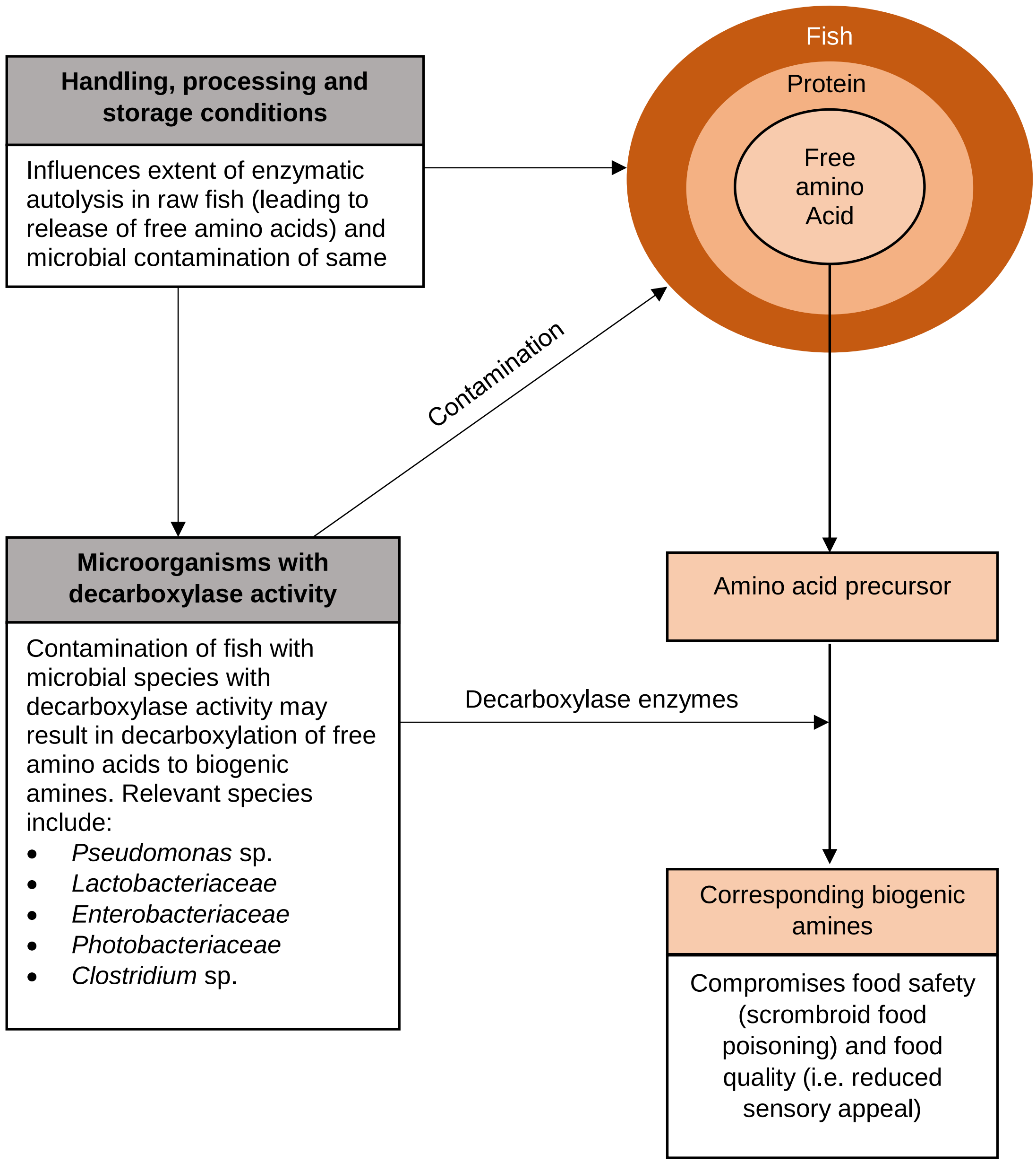
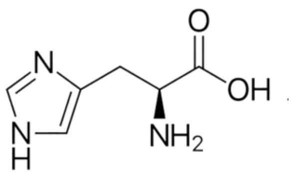
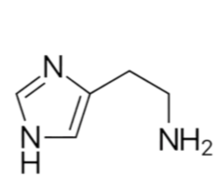
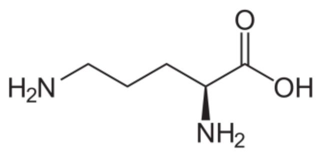
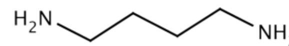
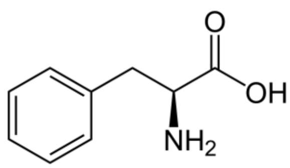
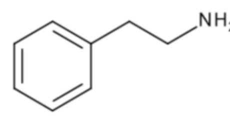
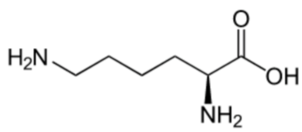
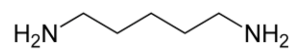
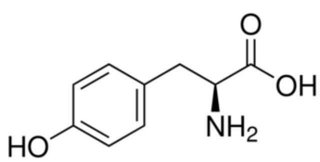
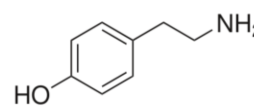
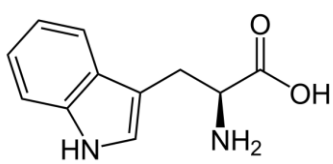
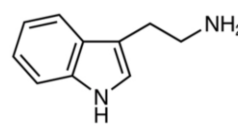


Fig . 1.10: A simple scheme for the formation of biogenic amines in fish (based on description in Ruiz-Capillas and Herrero, 2019)

Biogenic amines considered important in food safety and quality are histamine, putrescine, cadaverine, tyramine, tryptamine, ß-phenylethylamine, spermine, and spermidine (Shallaby, 1997). Table 1.2 shows the structures of some biogenic amines and their pre-cursor amino acids. Cadaverine and putrescine are especially relevant for their impact on the sensory quality of food; they give rise to a characteristic “dead body smell”. Ingestion of low amounts of biogenic amines may not be health-threatening because they are detoxified in the body by amine oxidases (Hu et al., 2012). However, some have been implicated in food poisoning episodes, causing symptoms such as respiratory distress, heart palpitations, headaches, oral burning, hypertension and hypotension (Becker *et al*., 2001).

Food poising linked to biogenic amines is called scombroid food poisoning (SFP), deriving its name from the groups of fish that were first implicated in the condition – *Scombridae* – such as tuna and mackerel (Ruiz-Capillas and Horrero, 2019). These fish are known to have high amounts of free histidine (Ruiz-Capillas and Moral, 2004). Other non-scombroid fish, such as sardines (*Sardinella* sp.), anchovies (*Engraulis* sp.) and herrings (*Clupea* sp.) have also been implicated in SFP (Hungerford, 2010).

Table 1.2: Some amino acids and their corresponding biogenic amines

Amino acid		Corresponding biogenic amine	
Name	**Structure**	**Name**	**Structure**
Histidine		Histamine	
Ornithine		Putrescine	
Phenylalanine		Phenylethylamine	
Lysine		Cadavarine	
Tyrosine		Tyramine	
Tryptophan		Tryptamine	

1.4.2.1 Regulation of biogenic amines

Codex identifies histamine as the most significant agent for SFP and also as the food safety indicator for biogenic amines (CAC, 2012). Between 1960 and 2005, histamine alone caused 16,000 cases of food intoxication in Europe (Leuschner et al., 2013). The Codex standard for histamine in foods is 200 mg/kg (CAC, 2012). National regulations, however, differ (Kim et al., 2009).

The Food and Drugs Administration of the United States (USFDA) uses limits of 50 mg/kg, 100 mg/kg and 1000 mg/kg for histamine, tyramine and total biogenic amines, respectively (Kim et al., 2009). In the EU, histamine is controlled with Regulation (EC) No. 1441/2008 (amendment of Regulation (EC) No. 2073/2005), with a focus on fish species having high histidine amounts. These include fish from the families *Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombresosidae* (European Commission, 2005, 2008). The criteria define histamine concentrations between 100 mg/kg (the limit separating good quality from marginally acceptable quality, denoted as "m") and 200 mg/kg (limit above which products are unacceptable, denoted as "M") as acceptable provided that, for every nine samples, only two have concentration values between "m" and "M" (Leuschner et al., 2013).

The characteristic unhygienic processing and handling conditions of traditional fish smoking in Ghana may favour contamination of the products with microbial genera such as *Pseudomonas* sp., *Vibrio* sp., *Enterobacteriaceae* and lactic acid bacteria that are known biogenic amine producers (Bunková et al., 2010; Zhai *et al*., 2011; Hu et al., 2012). The Ghana Standards Authority (GSA) adopts the EU standards for the management of histamine in fishery products (import and export) (GSA, 2014). Artisanal fish catches and traditionally processed fish on informal markets are not regulated for the hazard.

1.4.3 Polycyclic aromatic hydrocarbons (PAHs)

PAHs are a ubiquitous class of organic compounds with carcinogenic and mutagenic potential produced by incomplete combustion or exposure of complex organic substances to high temperatures or pressures (Ciecierska and Obiedziński 2007; Ravindra et al., 2008; Benford et al. 2010; Mičulis et al. 2011; Li et al., 2016; Singh et al., 2016; Howard and Fazio, 2018; Mohammadi and Valizadeh-kakhki, 2018). They are typically of anthropogenic origin, produced through activities such as burning wood, refuse, coal and petroleum products (Lerda, 2011).

Structurally, PAHs consist of two or more fused aromatic rings. Low molecular weight

PAHs have less than four rings (e.g. acenapthene, acenapthylene, fluorene and naphthalene), whereas the high molecular weight PAHs have four or more rings. Although all PAHs are generally lipophilic, those with high molecular weights have a higher octanol-water partition coefficient and are, thus, more lipophilic. According to Srogi (2007), aqueous solubility of the compounds reduces with increasing number of rings. This lipophilic character allows the compounds to bind to lipid membranes and get transported by lipoproteins in the blood (Ewa and Danuta, 2017). Some chemical and physical properties of PAHs are summarized in Table 1.3.

Table 1.3: Some chemical and physical properties of the 16 priority pollutant polycyclic aromatic hydrocarbons (*continued on next page*)

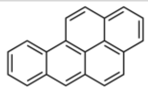
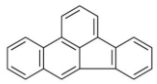
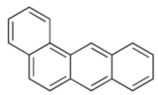
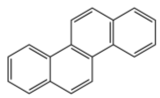
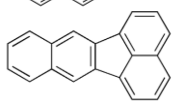
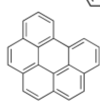
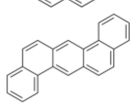
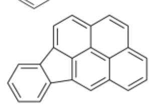
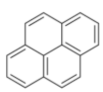
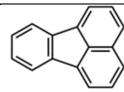
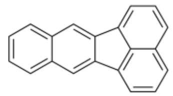
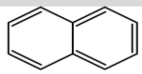
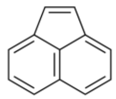
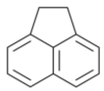
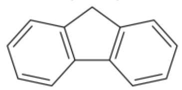
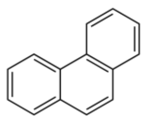
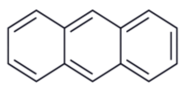
Compound	Structure	MW (g/mol)	Solubility in water (mg/L) at 25ºC	Vapour pressure (mm Hg)	log K_{ow}	log K_{oc}	Boiling point (ºC)	Melting point (ºC)	TEF	PLHS Rank
High molecular weight PAHs										
Benzo(a)pyrene*		252.3	0.001.6x10^{-3}	5.6x10^{-9}	6.04	6.74	311	179	1	8
Benzo(b)fluoranthene*		252.3	1.5x10^{-3}	5.0x10^{-7}	5.80	5.74	481	168	0.1	10
Benzo(a)anthracene*		228.3	9,4x10^{-3}	2.2x10^{-2}	5.91	6.14	437.6	158	0.1	38
Chrysene*		228.3	2.0 x 10^{-3}	6.3x10^{-9}	5.65	5.30	448	254	0.001	141
Benzo(k)fluoranthene		252.3	8.0 x 10^{-4}	5.1x10^{-7}	6.0	5.74	480	217	0.01	61
Benzo(g,h,i)perlyene		276.3	3.0 x 10^{-4}	1.0x10^{-10}	6.50	6.52	550	278	-	321
Dibenz(a,h)anthracene		278.4	2.0 x 10^{-3}	1.0x10^{-10}	6.75	6.20	524	262	1	15
Indeno(1,2,3-c,d)pyrene		276.3	6.9 x 10^{-4}	1.0x10^{-1}	7.66	6.20	536	164	0.1	174
Pyrene		202.3	1.3 x 10^{-1}	2.5x10^{-5}	5.18	4.58	404	150	-	255

Table 1.3: Some chemical and physical properties of the 16 priority pollutant polycyclic aromatic hydrocarbons (*continued from previous page*)

Fluoranthene		202.3	2.6×10^{-1}	5.0×10^{-6}	5.22	4.58	295	110.8	-	138
Benzo(k)flouranthene		252.3	8.0×10^{-4}	5.1×10^{-7}	6.0	5.74	480	217	0.01	61
					Low molecular weight PAHs					
Napthalene		128.1	3.1×10^{-1}	1.8×10^{-2}	3.37	-	217.9	80.26	-	80
Acenaphthylene		152.1	3.93	2.9×10^{-2}	4.0	3.40	280	91.8	-	343
Acenapthene		154.2	3.90	1.6×10^{-3}	3.92	3.65	279	93.6	-	168
Fluorene		166.2	1.69	7.1×10^{-4}	4.18	3.86	295	116	-	300
Phenanthrene		178.2	1.10	9.6×10^{-4}	4.57	4.15	340	101	-	248
Anthracene		178.2	4.3×10^{-2}	1.7×10^{-5}	4.54	4.15	340	216.4	-	306

*PAHs of food safety significance (EFSA, 2005)
**Solubility at 25ºC; no temperature reported for figures without asterisks
MW = molecular weight; Vp = vapour pressure (mm Hg); K_{ow} = octanol-water partitioning coefficient; K_{oc} = organic carbon partitioning coefficient; TEF = toxic equivalency factor; PLHS = priority list of hazardous substances. Principal data source: pubchem.ncbi.nlm.nih.gov

The main potential adverse health effect of PAHs in humans is cancer (Philips, 1999; Diggs et al., 2011; Lerda, 2011; Yebra-Pimentel et al., 2015; Zelinkova and Wenzl, 2015). This is related to the tendency of PAH metabolites to form adducts with DNA; hence they are genotoxic carcinogens (Yebra-Pimentel et al., 2015; Ewa and Danuta, 2017). The International Agency for Research on Cancer (IARC) identifies BaP as a human carcinogen (Group 1) and benzo(a)anthracene, chrysene, and benzo(b)fluoranthene as probable human carcinogens (Group 2B) (IARC, 2018). Human exposure occurs through inhalation (Kim et al., 2014), ingestion in food (Bansal and Kim, 2015), and dermal contact (Champmartin et al., 2017). Among these, food is the main route of exposure for non-smokers (Lerda, 2011).

The carcinogenic and mutagenic effects of BaP have been confirmed in animal studies. Ewa and Danuta (2017) report that exposure to BaP and/or its mixture caused immunotoxic, teratogenic effects, induced apoptosis and cell proliferation, and also increased DNA methylation. Different types of human cancers have been reported to have links with occupational exposure to PAHs in humans (IARC (2012). The specific occupational settings and the associated cancers are: (1) coke production—lung cancer, (2) coal gasification — lung and bladder, (3) paving and roofing— lung cancer, (4)coal-tar distillation — skin cancer, (5) soot — cancers of the lungs, oesophagus, haematolymphatic system and skin, (6) aluminum smelting — lung and bladder cancer, and (7) tobacco smoking —lung, lip, oral cavity, pharynx, oesophagus, larynx, and bladder cancers (IARC 2010, 2012).

In the human body, the liver is the main site for PAH metabolism (Chen et al., 2008). Two phases of PAH metabolism have been identified. The first phase involves a series of reactions including microsomal monooxygenations, cytosolic and mitochondrial oxidations, co-oxidations in the prostaglandin synthetase reactions, reductions, hydrolyses, and epoxide hydration (Ewa and Danuta, 2017). This phase is considered an activation stage and leads to the production of several intermediates (such as oxides of the parent PAH) that are more toxic than the parent compounds. BaP, for example, is an indirect mutagen. It is during the first phase of the metabolic reactions that it is transformed into active derivatives that have mutagenic properties.

The second phase is a detoxification process that results in the addition of endogenous substituents like sugars and free amino acids to the intermediates of phase one, thereby increasing their aqueous solubility and facilitating their excretion (ibid.) However, active derivatives form covalent bonds with DNA, with a potential to result in mutagenicity (Ewa and Danuta, 2017).

1.4.3.1 Regulation of PAHs in smoked fish

Codex has provided guidelines and normative instruments for controlling the occurrence of PAHs in smoked foods (CAC, 2009). However, Codex has not set limits for the hazards (Ingenbleek et al., 2019). In 2002, the Scientific Committee on Food (SCF) of the European Commission classified 15 PAHs as genotoxic both *in vitro* and *in vivo* (SCF, 2002). Subsequently, the Joint FAO/WHO Expert Committee on Food Additives concluded that 13 PAHs are carcinogenic in experimental animals, and benzo(a)pyrene (BaP) was identified as a marker for the hazard in foods (WHO, 2006).

In 2008, the European Food Safety Authority (EFSA) reported that BaP alone was not a suitable marker of PAH contamination in food, and concluded that the sum of BaP, benzo(a)anthracene, chrysene, and benzo(b)fluoranthene (PAH4) were an appropriate indicator (Ingenbleek et al., 2019). Regulation (EC) No. 835/2011 was then issued with maximum limits for BaP and PAH4 (2µg/kg and 12µg/kg, respectively), to replace Regulation (EC) No. 1881/2006 (European Commission, 2011).

In the absence of Codex standards on PAHs, the Ghana Standards Authority (GSA) adopts the EU regulations for the management of PAHs in smoked fish for export (GSA, 2014). In 2018, the authority launched its Code of Practice for the Reduction of Polycyclic Aromatic Hydrocarbons (PAHs) in Smoked Fish (GS 1131), in an attempt to translate Codex guidance documents to the local situation (GSA, 2018). At present, PAHs in smoked fish for domestic consumption are not regulated.

1.4.3.2 Occurrence of PAHs in traditionally smoked fish

During traditional hot smoking, smoke from the fuelwood coupled with the direct heat transfer results in contamination of the products with PAHs (Stolyhwo and Sikorski, 2005; Basak et al. 2010; Silva et al., 2011). Several studies have reported the occurrence of the hazards in smoked fish in Africa. The first multi-centre sub-Saharan Africa Total Diet Study on PAHs found a 100% occurrence of the hazard in smoked fish, and 100% exceedance of the EU regulatory limits (Ingenbleek et al., 2019). The study also reported that smoked fish had the highest mean PAH4 contamination level (180 µg/kg) among the food groups tested (Ingenbleek et al., 2019).

Mahugija and Njale (2018) reported mean levels of 390 µg/kg in smoked fish in Tanzania. In Ghana, Palm et al., (2011) and Essumang et al., (2012, 2013, 2014) reported levels in excess of 19 times the EU limits in smoked fish on informal markets. Similar findings have also been made in neighbouring Nigeria (Olabemiwo et al, .2011; Akpambang et al., 2009). A summary of such studies is presented in Table 1.4, all of which highlight the traditional smoking process as the root cause of the elevated hazard contamination levels.

Along with Cote d'Ivoire, Cameroun, and Nigeria, Ghana is a major exporter of smoked fish to the EU, mainly targeted at the diaspora (Ward, 2003). Product detention at ports of entry are not uncommon. Several notifications of the rapid alert system for food and feed (RASFF) on smoked fish from Africa have been due to unacceptable PAH levels in the products. In the United Kingdom, it has been estimated that about 70% of detained consignments are destroyed (Ward, 2009). These product rejections due to unsatisfactory safety quality of the fish products could be considered a post-harvest loss (Diei-Ouadi and Mgawe, 2011) and, indeed, constitute a direct economic loss to the exporters.

Table 1.4: Studies on the occurrence of polycyclic aromatic hydrocarbons in smoked fish in Africa

Country	Fish species	No. of PAHs analysed	Reported concentration of PAHs	Analytical method (as described in reference)	Reference
Nigeria	*Clarias gariepinus, Pseudotolithus senegalensis, Scomber scombrus and Selar crumenophthalmus*	16	BaP: 31.2 µg/kg (max)	HPLC with fluorescence detection. Column was c18 reversed phase Supelcosil LC-PAH	Akpambang et al., 2009
Ghana	*Sardinella* sp.	16	BaP: 73.8 µg/kg (max) ∑PAH: 511 µg/kg – 1416.8 µg/kg	GC-MS and VF-5 ms fused capillary column. Splitless injection mode.	Essumang et al., 2012
Uganda	*Lates niloticus*	9	∑PAH: 23.4 – 58.1 µg/kg	GC-MS and Zebron ZB-5MSi column. Splitless injection mode.	Ongwech et al., 2013
Nigeria	*Clarias gariepinus, Arius heudeloti, Cynoglussus senegalensis, Mud minnow and Blunt hwake*	16	BaP: 5.4 – 5.7 µg/kg	GC-MS	Ubwa et al., 2015
	Tilapia spp., Arius heudeloti and others	16	BaP: 2400 µg/kg ∑PAH: 79700 µg/kg	GC-MS	Okenyi et al., 2016
	Clarias gariepinus, Tilapia zilli, Ethmalosa fimbriata, and Scomber scombrus	16	BaP 288 µg/kg ∑PAH: 3590µg/kg	GC-FID. Splitless injection	Tango et al., 2017
Tanzania	*S. victoria, Haplochromis spp and L. niloticus*	13	BaP: 390 – 1550 µg/kg ∑PAHs: 33900 µg/kg	GC-MS on Rtx-5MS column. Splitless injection mode.	Mahugija and Njale, 2018
Benin	Unspecified pool of species available on the market	16	BaP: 3.41 – 77.5 µg/kg PAH4: 30.6 – 560.4 µg /kg PAH16: 55.2 – 984.7µg/kg	GC-MS/MS. Splitless injection mode. Agilent Select PAH column	Ingenbleek et al., 2019
Cameroun	Unspecified pool of species available on the market	16	BaP: 10.5 – 32.5 µg/kg PAH4: 65.2 – 198.4 µg/kg PAH16: 125.1 – 330.4 µg/kg	GC-MS/MS. Splitless injection mode. Agilent Select PAH column	Ingenbleek et al. 2019
Mali	Unspecified pool of species available on the market	16	BaP: 16.7 – 17.4 µg/kg PAH4: 109.4 – 114 µg/kg PAH16: 178.7 – 186.7µg/kg	GC-MS/MS. Splitless injection mode. Agilent Select PAH column	Ingenbleek et al., 2019

1.5 Lipid oxidation in (smoked) fish

Fish is a known rich source of unsaturated fatty acids, especially the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kris-Etherton et al., 2002). As a result, lipids in fish are highly susceptible to oxidation, (Mozuraityte et al., 2016). Since thermal processing accelerates such oxidation (Schaich, 2005; Frankel, 2012), the stability of lipids in the smoked fish produced in Ghana may be minimal, with consequences for the sensory, nutritional and safety quality of the products (Cropotova et al., 2019). Oxidized lipids may produce off-flavours (reduced sensory quality), diminish the unsaturated fatty acid content (loss in nutritional value) and the oxidation products may be injurious to health (compromised food safety). In addition to the thermal treatment of smoking, the traditional practice of storing smoked fish under ambient tropical temperatures may further promote the oxidation.

Fundamentally, lipid oxidation occurs through three steps: initiation, propagation and termination (Frankel, 2012; Schaich, 2005). The initiation step involves abstraction of a hydrogen atom from an unsaturated fatty acid to form a free alkyl radical, which then reacts with oxygen to form a peroxy radical. A series of radical-forming reactions then proceeds until the formation of non-radical products terminates the reaction, as summarized in Fig. 1.11.

The primary products of lipid oxidation are hydroperoxides, which upon decomposition form secondary products such as aldehydes, ketones and oxo-fatty acids (Belitz et al., 2009). The range of secondary oxidation products of lipids is extensive (Cropotova et al., 2019). Frankel (2012) reports that more than 150 of such compounds were found in oxidized fish oil. This complicates the measurement of the oxidation status of lipids and the interpretation of results obtained. Conventionally, lipid oxidation is determined by measuring the peroxide value and levels of secondary oxidation products such as hexanal, malondialdehyde, 4-hydroxyhex-2-enal and 4-hydroxynon-2-enal (Obando et al., 2015; Vandermoortele, 2020). It has been shown, however, that these measurements may not accurately reflect the oxidative state of the lipids. Malondialdehyde, for example, has high reactivity and could thus be found in low concentrations in highly oxidized lipids, thus raising questions about the suitability of the traditional lipid oxidation markers (Vandemoortele et al., 2015; 2017).

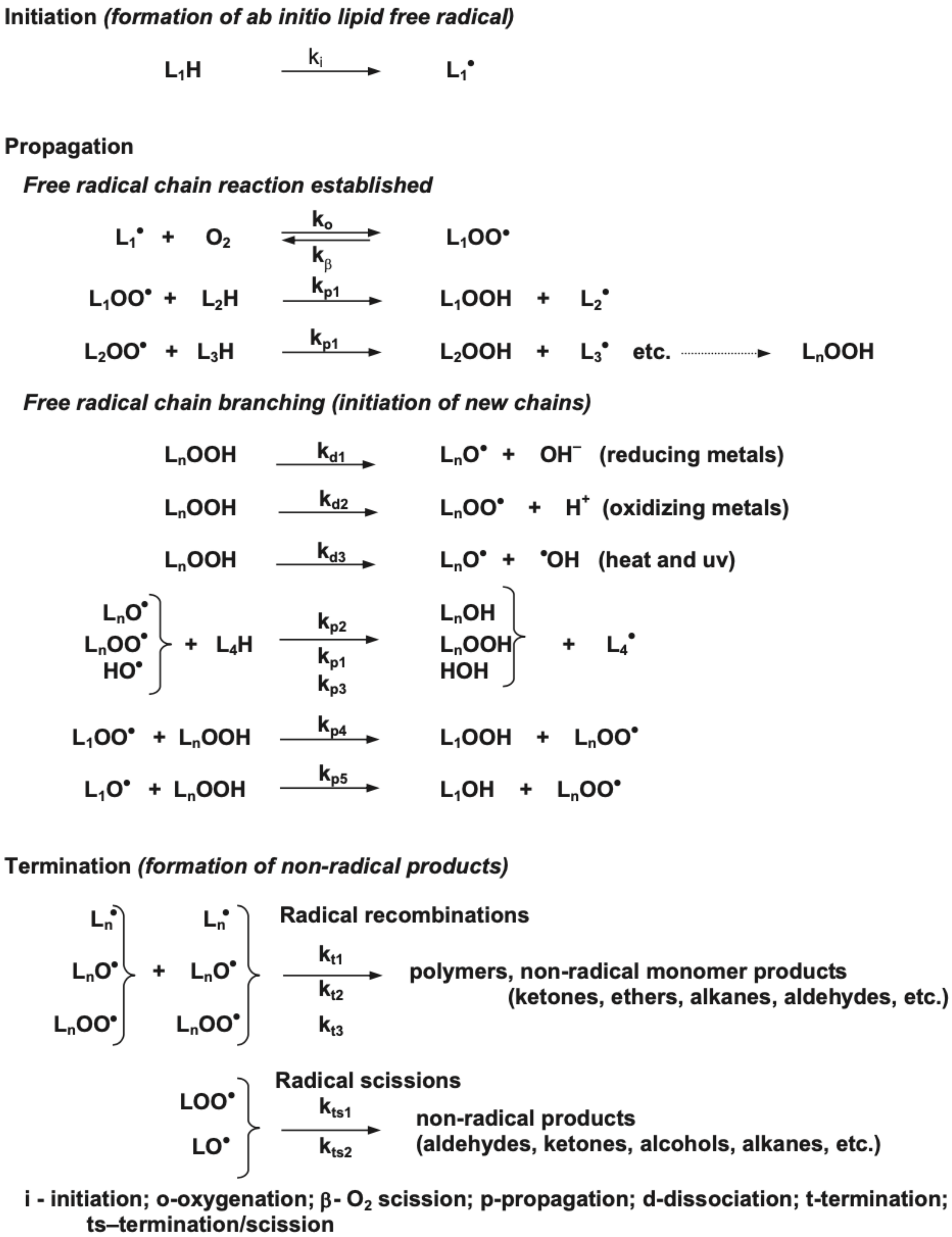


Fig. 1.11: Traditional radical chain reaction of lipid oxidation (from Schaich, 2005).

Additionally, the secondary oxidation products result from the degradation of alkoxy radicals of fatty acids. The remaining part of the modified, oxidized fatty acids are rarely considered, although they may have implications for food safety. These include compounds such as oxo-fatty acids, hydroxy fatty acids and epoxy fatty acids (Berdeaux et al., 2009).

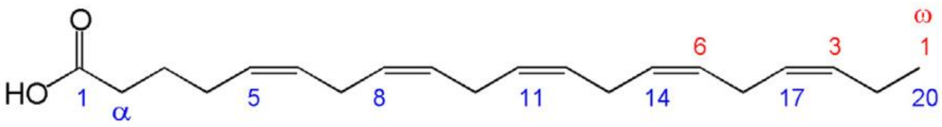
1.5.1 Potential occurrence of epoxy fatty acids in fish lipids and potential use as oxidation indicators

Epoxy fatty acids form by a replacement of one or more double bonds of an unsaturated fatty acid with an oxirane ring (a three-membered, cyclic carbon-oxygen-carbon ring) (Chow, 2007). Due to the high reactivity of the oxirane ring, epoxides have the potential to react with DNA and proteins to cause structural damage and functionality alteration that could result in mutagenicity (Greene et al., 2000). Animal studies have shown that epoxy fatty acids are toxic in rats (Le Quéré et al., 2004) and have been associated with maladies such as organ failure, cancer and reproductive defects in those experimental animals (Goicoechea and Guillen, 2010).

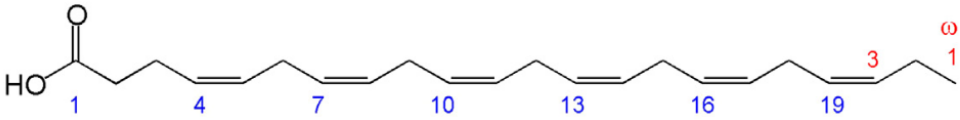
Since epoxy fatty acids result from lipid oxidation, it would be expected that their levels will correspond with the extent of oxidation (compound stability granted). However, Mubiru et al. (2013) found that in vegetable oils with low peroxide values (<12.6 meq O_2 per g of oil, indicating low level of oxidation), unexpectedly high amounts of epoxy fatty acids were present (up to 2 mg per g of oil). This implies that the amounts of the epoxides may in fact be a more sensitive indicator of oxidation than the peroxide value.

At the time of this study, no reports were found in the available scientific literature on the occurrence of epoxy fatty acids in fish as such nor in fish oil. Considering the high susceptibility of fish lipids to oxidation due to the predominance of unsaturated fatty acids, it is probable that epoxides could be present when such lipids are oxidized. In the case of the smoked fish, the heat exposure and subsequent traditional method of storage may increase that probability. It is therefore worth investigating their occurrence and potential use as markers of oxidation in (smoked) fish lipids.

Fig. 1.12 shows the structure of the omega-3 unsaturated fatty acids EPA and DHA (the dominant polyunsaturated fatty acids in fish lipids). The full set of monoepoxy fatty acids (i.e. containing only one epoxy group) that can be derived from these two fatty acids are provided in Tables 1.5 and 1.6.



all-cis-5,8,11,14,17-eicosapentaenoic acid (EPA)



all-cis-4,7,10,13,16,19-docosahexaenoic acid (DHA)

Fig. 1.12: Structure of long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), showing the numbering of carbon atoms from the alpha (α) and omega (ω) ends

Table 1.5: Derived epoxides of 5,8,11,14,17-eicosapentaenoic acid (EPA)

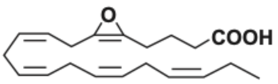
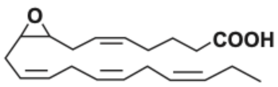
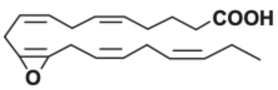
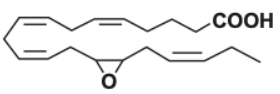
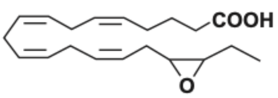
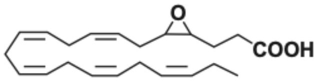
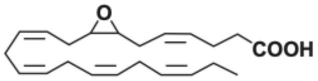
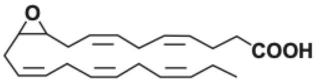
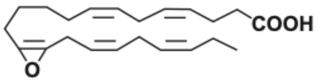
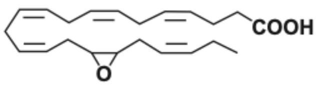
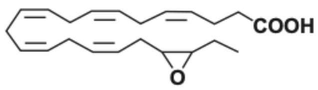
Structure	Name
	5,6-epoxy-8,11,14,17-eicosatetraenoate
	8,9-epoxy-5,11,14,17-eicosatetraenoate
	11,12-epoxy-5,8,14,17-eicosatetraenoate
	14,15-epoxy-5,8,11,17-eicosatetraenoate
	17,18-epoxy-5,8,11,14-eicosatetraenoate

Table 1.6: Derived epoxides of 4,7,10,13,16,19-docosahexaenoic acid (DHA)

Structure	Name
	4,5-epoxy-7,10,13,16,19-docosapentaenoate
	7,8-epoxy-4,10,13,16,19-docosapentaenoate
	10,11-epoxy-4,7,13,16,19-docosapentaenoate
	13,14-epoxy-4,7,10,16,19-docosapentaenoate
	16,17-epoxy-4,7,10,13,19-docosapentaenoate
	19,20-epoxy-4,7,10,13,16-docosapentaenoate

1.6 The challenge of multiple hazards in one food commodity

As seen in the preceding sections, several confirmed and potential hazards are associated with traditionally smoked fish. Without scientific evidence on the relevance of each hazard in a national context, there may be inefficient use of resources through unguided actions on food safety management. Given the significant part smoked fish occupies in the Ghanaian diet, it is important to clearly identify which hazards should be prioritized for food safety management, in order to select and implement appropriate strategies to minimize the impact of the hazards on public health. This is done within the broader framework of food safety risk analysis.

According to the Codex, in food safety, risk is a function of the likelihood of occurrence of a hazard in food, and the severity of the adverse health effect on human health upon exposure to that hazard (CAC, 2001). Risk analysis seeks to evaluate the impact of hazards on public health, identify appropriate mitigation strategies and maintain an on-going transparent exchange of reliable information among stakeholders as a shared responsibility, to prevent the occurrence and subsequent human exposure to the hazards (FAO, 2005).

There are three distinct yet connected components of food safety risk analysis, namely risk assessment, risk management and risk communication (FAO 2005; FAO, 2020) (Fig. 1.13).

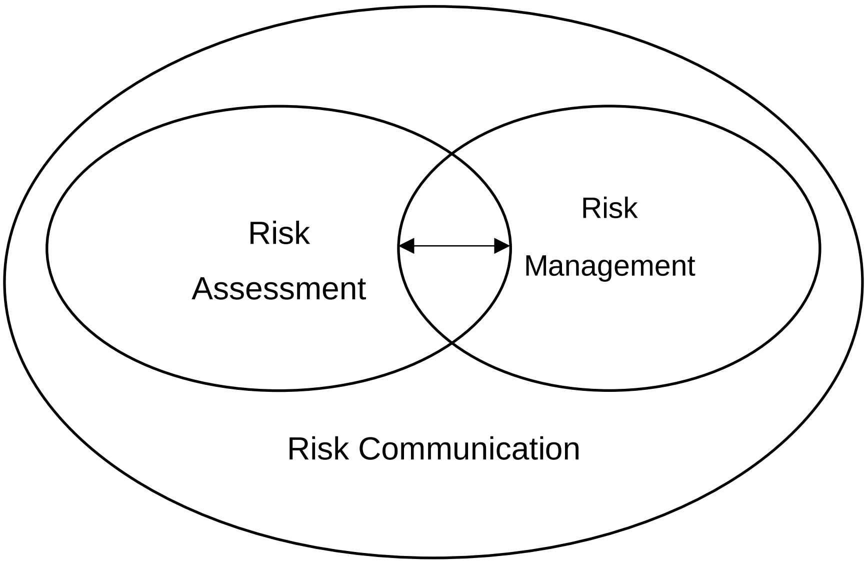


Fig. 1.13: Food safety risk analysis framework (CAC, 1999)

1.6.1 Risk assessment

Risk assessment is considered the central component of food safety risk analysis (FAO/WHO, 2009). It provides the scientific basis for the other two components of the risk analysis framework, since its output is used for the establishment, implementation and continuous improvement of appropriate preventive and control measures for food safety risks (risk management) and for a continual dialogue among stakeholders concerning the risk (risk communication) (CAC, 2003). According to the Codex, risk assessment consists of hazard identification, hazard characterization, exposure assessment and risk characterisation (CAC, 2015). These steps are summarized in Fig. 1.14., in which, for each step, a question indicates the primary interest and the bullet points indicate the inputs required to meet the interests.

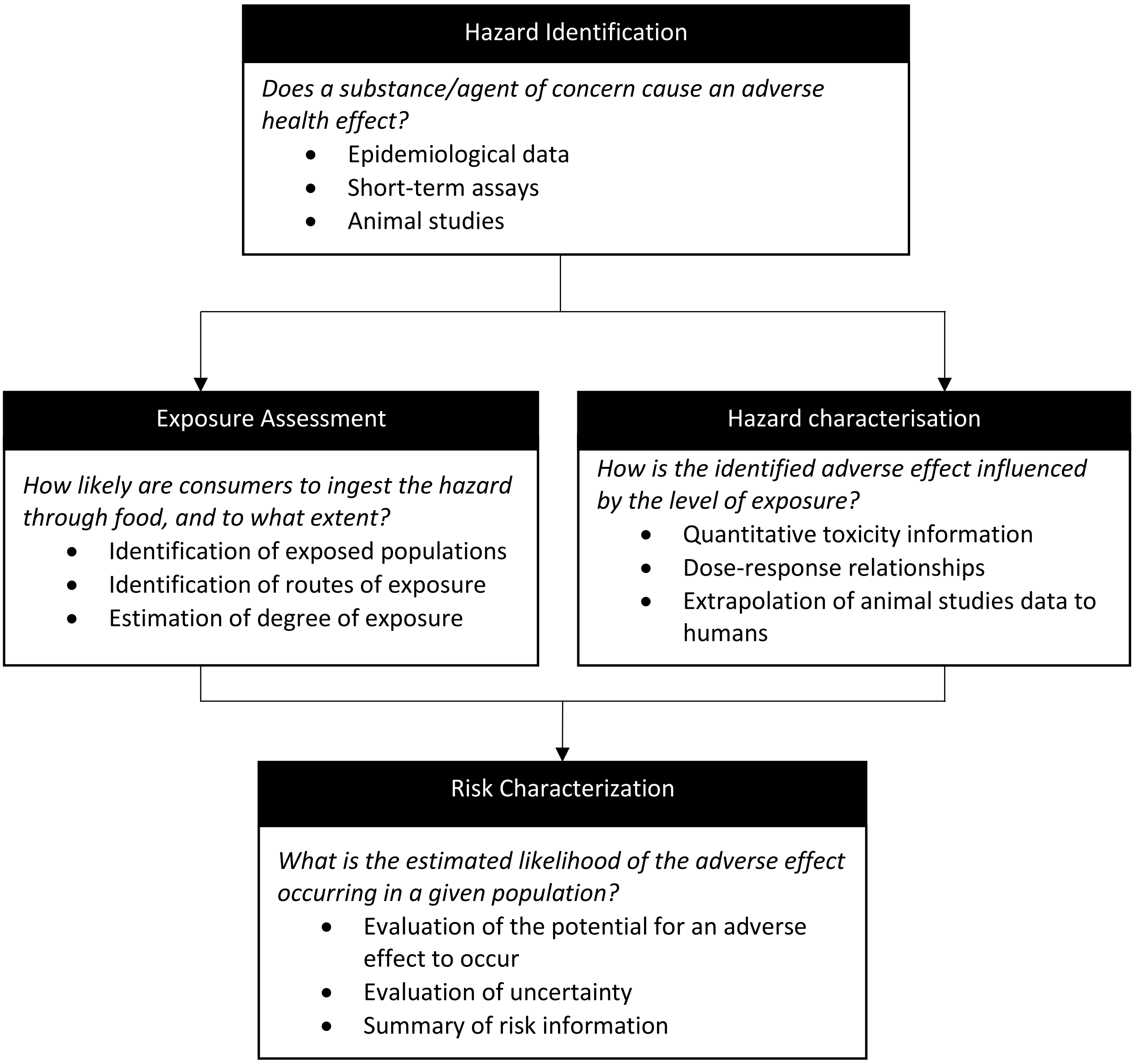


Fig. 1.14: Elements of food safety risk assessment (Derelanko and Hollinger, 2001)

1.6.1.1 Hazard identification

The hazard identification step focuses on identifying biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods (CAC, 2015). It requires determination of the known or potential adverse health effects associated with a particular hazard (Brown, 2002), through an in-depth review of available scientific information regarding the nature, occurrence (association with foods), and mechanisms of action of hazards that influence their toxicity.

The validity of the entire risk assessment process depends on the adequacy of the hazard identification step. It is important to ensure that declared hazard-food associations are realistic, both on the basis of empirical data and from experience on food production, processing, handling and consumption practices (Lammerding and Fazil, 2000). Generating a tall list of *possible* hazards without considering if they are likely to affect consumer health can make the risk assessment process cumbersome and should be avoided. Similarly, failing to list all hazards that are *relevant* (i.e. science-supported association between the hazard and the food, with potential to cause adverse health effects) to the product should also be avoided, as this will compromise the exhaustiveness of risk management actions (Brown, 2002).

In general, hazard identification should capture information including but not limited to characteristics of the hazard(s) and the food(s) in which they occur and how these characteristics favour their association, estimates of the concentration and prevalence of the hazard in the food(s) concerned, influence of food processing and/or preparation on the occurrence of the hazards, evidence of causal link between hazard and adverse health effect in humans (epidemiological data), toxicity/virulence attributes and host susceptibility factors as well as consumer profile (e.g. demographic affected, patterns of food consumption, food handling/preparation practices) (Brown, 2002; Heggum, 2011). Table 1.7 shows a number of criteria that may be applied to identify relevant hazards in food.

Table 1.7: Assessing causal link between a food and/or hazard and illness

Criteria	Definition
Strength	There is a strong relationship between exposure to the food-hazard pair and acute and/or chronic illness (e.g. outbreaks, case-control studies).
Specificity	There is evidence that a specific population developed illness after exposure and there is no other plausible explanation for the cause of the illness.
Temporality	There is evidence that exposure to a food-hazard pair precedes acute and/or chronic illness (e.g. outbreaks, sporadic cases, cohort studies)
Dose-response	There is evidence of a direct relationship between increasing levels of hazard exposure and the risk of acute and/or chronic illness.
Plausibility	It is biologically plausible that the hazard can occur in the food and/or cause acute and/or chronic illness in humans.
Coherence	The food-hazard pair "makes sense" given current knowledge about the food supply and food safety.
Experimental evidence	There is experimental evidence suggesting that hazard exposure causes acute and/or chronic illness (e.g. animal models) or that the hazard can occur in the food (prevalence studies).
Analogy	The food supports the growth/maintenance of a similar hazard (e.g. if STEC O157 is a known hazard for food, then STEC non-O157 should also be considered; for chemicals, if one metal is identified in foods, it is possible that other metals are also present) or a hazard is associated with a similar food (e.g. *Cyclospora* in raspberries and strawberries; benzene may be found in butter or cheese).
Specificity	There is evidence that a specific population developed illness after exposure and there is no other plausible explanation for the cause of the illness.

Source: FAO, 2020

1.6.1.2 Exposure assessment

The exposure assessment step covers a qualitative and/or quantitative evaluation of the likely intake of hazards via foods as well as exposures from other sources, if relevant (CAC, 2015). It measures how much of a given hazard is likely to be ingested through food by a given (sub-group of a) population. The main inputs are the (estimated) hazard levels in the product at the point of consumption and the quantity of food consumed. According to Heggum (2014), some factors to consider in exposure assessments include:

1. The prevalence and concentration of hazards in food, as influenced by
    a. characteristics of the hazard
    b. the nature and ecology of the food (for microbial hazards)
    c. the initial contamination of the raw material
    d. the level of process controls, if any
    e. the methods of processing, packaging, distribution and storage
2. Food consumption habits as influenced by socioeconomic and cultural backgrounds, ethnicity, seasonality of food, population demographics and consumer preferences and behaviour.

Concerning food consumption habits, information such as the typical serving sizes, frequency of consumption (daily, weekly or annual consumption rate), and food preparation practices are needed. Additionally, information on vulnerable groups (the young, old, pregnant, immunocompromised and malnourished (YOPIM)) should be provided, as these groups have different eating habits and are more susceptible to the adverse effects of exposure.

Information sources for exposure assessment include primary research data, published literature, food surveillance repositories and expert consultation, among others. Data availability may not be sufficient for the demands of the exposure assessment (and, indeed, the overall risk assessment). Hence, the uncertainties and variabilities associated with the estimates and their potential impact on the interpretation of the results should be transparently communicated. Variability is an inherent heterogeneity in data, whereas uncertainty refers to gaps in knowledge. Whiles obtaining more information (e.g. closing data gaps) may reduce uncertainty, additional data does not decrease variability (Tran et al., 2006).

Where determined quantitatively, outputs of exposure assessments should clearly state the amount of hazards ingested by consumers per unit quantity of food (Brown, 2002). This output could be deterministic (i.e. based on single values of exposure variables, e.g. using only the mean hazard concentration and mean quantity of food consumed to estimate exposure) or probabilistic (using distribution functions for each exposure variable). Deterministic exposure calculations could lead to under- or overestimation of exposure in the population. Use of the distribution functions allows consideration of variabilities and uncertainties in the exposure inputs (Tran et al., 2006).

1.6.1.3 Hazard characterization

Hazard characterisation is a qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with hazards (CAC, 2015). It is a description of the relationship between the levels of a hazard ingested in food (dose) and the probability and severity of its adverse health effects (response) (Dennis et al., 2002). Due to the variabilities in vulnerability in human populations, dose-response relationships differ for different sub-populations. Differences in age, gender, and genetics affect susceptibility of individuals to illness, and may thus require different dose-response relationships to describe the adverse health effects that may result from exposure (Dennis et al., 2002).

In broad terms, two models of dose-response relationships have been described, namely threshold model and non-threshold model. The former assumes that there is a hazard dose level up to which some consumers can be exposed without suffering adverse health effects, whiles the latter assumes that all hazard doses can potentially cause an adverse health effect (FAO/WHO, 2009). For microbial hazards, the threshold model assumes that a minimum infectious dose of pathogens is required to make a consumer sick, whereas the non-threshold model assumes that a single cell can cause illness. For chemical hazards, non-carcinogenic hazards generally follow the threshold-model, whereas carcinogenic hazards follow the non-threshold model (Dennis et al., 2002).

For the threshold dose-response model for chemical hazards, the thresholds considered are the lowest observed-adverse-effect level (LOAEL) and the no-observed-adverse-effect level (NOAEL). The former refers to the lowest amount of a given hazard that causes a measurable adverse effect, while the latter refers to the highest amount of the hazard which produced no measureable adverse effect in the most sensitive experimental subject (FAO/WHO, 2009). Based on NOAEL, health-based guidance values such as acceptable daily intake (ADI) and tolerable daily intake (TDI) can be calculated for non-carcinogenic compounds. These guidance values represent exposure levels considered not to present an appreciable health risk. TDI is used for food contaminants and ADI for substances with controllable exposures (e.g. food additives, pesticide residues and veterinary drugs in foods) (FAO/WHO, 2009).

1.6.1.4 Risk characterization

The Codex defines risk characterisation as the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment (CAC, 2015). It is the integration of the outputs of the previous steps to arrive at a qualitative statement of risk (e.g. low, medium, high) or a quantitative estimate (e.g. number of cancer incidents per year within the (sub)population of interest), including a description of the uncertainties and variabilities inherent in the entire risk assessment exercise (FAO/WHO, 2009; Brown, 2002).

1.6.1.5 Types of risk assessment

In relation to hazards, two principal types of risk assessments are identified: chemical risk assessment, focusing on chemical hazards, and microbiological risk assessment, focusing on microbiological hazards. The four steps discussed above are applied in either type of risk assessment. According to Heggum (2014), risk assessment can also be applied to physical hazards in foods.

In relation to the desired risk output, risk assessment can be qualitative (provides descriptive categories of risk, e.g. low, medium high), quantitative (provides numerical estimates of risk), or semi-quantitative (evaluating risks with scores).

1.6.2 Risk management

Risk management is the political arm of risk analysis that translates the results of risk assessments into actions, guided by governance priorities (FAO/WHO, 2009). The Codex defines risk management as the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options (CAC, 2015).

Four steps are identified for the risk management process, namely, risk evaluation, risk management options assessment, implementation, and monitoring and review (Heggum, 2014). The risk evaluation determines the scope of risk assessments and also evaluates their outcomes. The evaluation may involve ranking assessed hazards based on their respective risk estimates, in order to support priority setting and its attendant resource allocation. The assessment of risk management options typically covers identification of the available management options, selection of a preferred option, evaluation of the impact of the preferred option on other factors (e.g. economic, social, and political impact), and a confirmation of a final decision (Heggum, 2014). Following implementation of the final decision, it is continually monitored and reviewed to measure its effectiveness to determine if, and what, further measures may be needed to safeguard public health.

The risk evaluation feature of ranking hazards is an important exercise that enables risk-based priority setting in support of efficient resource allocation and use (Van der Fels-Klerx et al., 2018). This is discussed next.

1.6.2.1 Risk ranking

Risk ranking is an important activity in risk management that involves an analysis and ordering of identified hazards and/or foods to establish their relative importance vis-à-vis the magnitude of their impact on public health (FAO, 2020). Such an ordering provides a transparent and practical basis that informs the nature, timing and resource needs of mitigation strategies (Van Kreijl et al., 2006).

Van der Fels-Klerx et al., 2018 critically reviewed available methods for risk ranking and reported several approaches in use, ranging from qualitative through semi-quantitative to quantitative methods. The characteristics of the available methods are summarized in Table 1.8.

Table 1.8: Characteristics of food safety risk ranking methods

Characteristic	Risk ranking method										
	Risk assessment	**Comparative risk assessment**	**Ratio (exposure/ effect)**	**Scoring method**	**Cost of illness**	**HALY***	**WTP***	**MCDA***	**Risk matrix**	**Flowcharts /decision trees**	**Expert synthesis**
A. CHARACTERISTICS											
1. Amount of resources (time, money)	High	High	Moderate	Moderate	Moderate	Moderate	High	High	Low	Low	Moderate/Low
2. Level of output	Quantitative	Quantitative	Semi-quantitative	Semi-quantitative	(Semi-) quantitative	(Semi-) quantitative	(Semi-) quantitative	(Semi-) quantitati ve	Qualitative/ semi-quantitative	Qualitative	Qualitative
3. Easy to explain to stakeholders (laymen)?	No	No	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes
4. Inclusion stakeholder perception	Not possible	Not possible	Not possible	Possible	Not possible	Not possible	Possible	Possible	Not possible	Possible	Possible
5. Inclusion of uncertainty	Possible	Possible	Possible	Possible	Possible	Possible	Possible	Possible	Not possible	Not possible	Possible
6. Inclusion of weights for the risk ranking criteria	Not possible	Not possible	Not possible	Possible	Not possible	Not possible	Not possible	Possible	Not possible	Not possible	Not possible
7. Inclusion human incidences	Possible	Possible	Not possible	Not possible	Possible	Possible	Possible	Possible	Not possible	Possible	Possible
8. Inclusion economic impact	Not possible	Not possible	Not possible	Not possible	Possible	Not possible	Possible	Possible	Not possible	Possible	Possible
9. Common method of communication (in addition to reports)	Graphs/ Tables	Graphs/ Tables	Tables	Tables	Graphs/Tables	Graphs/Tables	Graphs/ Tables	Graphs/ Tables	Graphs	Decision tree	Tables
B. ESSENTIAL DATA NEEDED											
1. Human incidence data needed?	No	Yes	No	No	Yes	Yes	Yes	No	No	No	No
2. Dose-response data needed?	Yes	Yes	No	No	No	No	No	No	No	No	No
3. Occurrence data (concentration, prevalence, dose) needed?	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No
4. Food consumption data needed?	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No
5. Growth models needed (only applicable for microbiological hazards)?	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No
6. Toxicological reference values (ADI, TDI, etc.) needed (only for chemical hazards)?	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No

Source: Van der Fels-Klerx et al., 2018. *HALY = health adjusted life years; WTP = willingness to pay; MCDA = multi criteria decision analysis

FAO (2020) proposes a three-step approach for conducting a risk ranking. These steps are:

a) definition of the scope of the risk ranking, in which the purpose of the exercise is clarified, what needs to be ranked is selected (foods or hazards or both), and the foods and/or hazards screened for overall relevance and risk potential,
b) development of the approach, covering selection of the risk ranking method (see Table 1.8), selection of the metrics for ranking the risks, and collection and evaluation of the appropriateness of data, and
c) conducting the risk ranking with the selected method and reporting the results.

Fig.1.15 summarizes several situations that could constitute the focus of risk ranking.

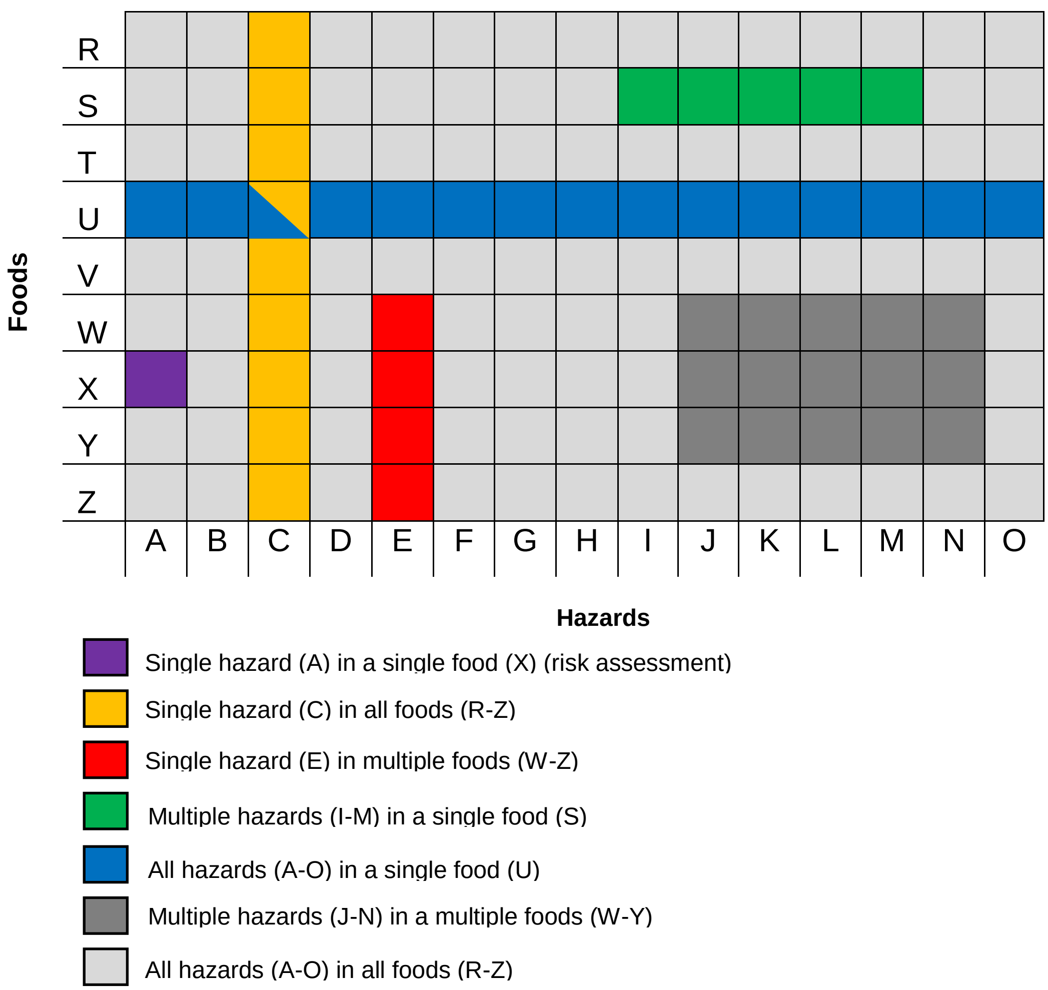


Fig. 1.15: Hypothetical representation of the possible focus of a risk ranking (FAO, 2020)

It is the responsibility of the risk manager to clarify the foods and hazards of interest to guide the risk ranking activity. This selection is informed by concerns such as incidence/increasing rates of foodborne disease outbreaks and non-compliance alerts in traded food commodities. The selection typically involves appropriate (technical) consultations that ensure that hazards relevant to the context (e.g. peculiar food production, handling and consumption habits and risk ranking goal) are not missed.

Havelaar et al., (2012) used risk ranking to determine the disease burden of foodborne pathogens in the Netherlands, using disability adjusted life years (DALYs, a measure of the years of life lost due to illness). They evaluated a total of 14 pathogens, comprising seven agents for infectious gastroenteritis (thermophilic *Campylobacter* spp., Shiga-toxin producing *Escherichia coli* O157 (STEC O157), nontyphoidal *Salmonella* sp., norovirus, rotavirus, *Cryptosporidium* sp., *Giardia* spp.), three agents of microbial intoxication (*Bacillus cereus, Clostridium perfringens and Staphylococcus aureus*) and four pathogens that cause systemic infections (*Listeria monocytogenes*, hepatitis A virus, hepatitis E virus, and *Toxoplasma gondii*). Food and non-food routes of exposure were considered. Food accounted for 50% of the transmission of the pathogens, indicating that food was the most important exposure route requiring risk management action. Among the 14 hazards, *T. gondii* and *Campylobacter* spp. accounted for the highest foodborne disease burden on a population basis, whereas, at the individual level, *T. Gondi* and *L. monocytogenes* were the pathogens with the highest burden. This kind of output enables streamlining of risk management actions, as it highlights the most important hazards and exposure routes as far as public health is concerned.

Anderson et al., (2011) also ranked the relative public health impact of pathogen-produce combinations in the United States. Among a total of 53 fruits and vegetables, each paired with a relevant pathogen, *E. coli* in leafy greens ranked as most important, followed by *Salmonella* sp. in tomatoes and *Salmonella* sp. in leafy greens, thus highlighting the relevant areas of focus for further risk assessment (to determine actual extent of risk posed by each hazard) and/or risk management actions. A list of some other risk ranking studies is provided in Table 1.9.

Table 1.9: Examples of risk ranking studies/efforts highlighting the hazard-food combination of interest, the risk ranking objectives, assessment tool and related metric

Food(s)	Hazard(s)	Risk ranking objective	Method or tool used	Metric	Reference
Foods of animal origin	Licensed veterinary medicinal products and medicated feed additives	Develop a national residue control plan for chemical residues and contaminants in food/feed	Semi-quantitative MCDA	Score	FSAI (2014)
All imported foods	Microbial and chemical	Identify high-risk imported foods to target for further examination	Quantitative (Tool: Predictive Risk-based Evaluation for Dynamic Import Compliance Targeting [PREDICT])	Score	GAO (2016)
30 food sector categories	Microbial and toxins	Identify high risk food sectors to prioritize for implementation of food control plans	Semi-quantitative: MCDA	Score	NZFSA (2006)
Pork	Microbial and chemical (including antibiotics)	Identify and rank the main risks for public health that should be addressed by meat inspection for swine	Qualitative	Qualitative	EFSA (2011)
Multiple food categories	Pesticide residues	Identify highest risk pesticide residues in foods to the consumer	Quantitative: public health criteria	Score	Low et al. (2004)
Beef, sheep, goat meat	Microbial	Identify high risk foods in the red meat industry for prioritizing risk management actions	Qualitative and semi-quantitative (Tool: Risk Ranger)	Score	Sumner et al., 2015
12 food categories	14 foodborne pathogens	Estimate of the disease burden of foodborne pathogens in the US	Quantitative: top-down	QALYs	Batz et al. 2012
30 food types representing 7 food groups	Acrylamide	Estimate the burden of disease caused by dietary exposure to acrylamide	Quantitative: bottom-up	DALYs	Jakobsen et al. 2016

Source: FAO, 2020

1.6.3 Risk communication

This is the component that holds the risk analysis framework together. It is the springboard for risk assessment and risk management, serving as the medium by which either is commissioned and sustained. The formal definition of risk communication is the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions (CAC, 2015).

The aim of risk communication is to promote understanding and dialogue among stakeholders about decisions concerning the management of food safety risks, and to help consumers make informed judgements about the food safety hazards and risks (EFSA, 2012). According to FAO/WHO (1998), risk communication is expected to:

a. involve and inform all interested parties within the risk analysis process
b. assist the development of transparent and credible decision-making processes related to food safety risks
c. instil confidence in all stakeholders about risk management decisions, and garner their support for same

Risk communication strengthens the effectiveness of risk management programmes by equipping consumers with useful information about the risks associated with a food product to enable them to use/consume it safely; increasing public awareness of the nature of a food safety risks, and providing fair, accurate and appropriate information to enable consumers make informed choices on what suits their risk tolerance (EFSA (2012).

EFSA identifies openness, transparency, independence and responsiveness as important pillars for an effective risk communication (EFSA, 2012). *Openness* refers to the free and timely release of information based on which risk management decisions are made, to allow stakeholder scrutiny of same; *transparency* concerns the clear communication of all uncertainties in the risk assessment, their potential impact on the risk management decision, and the ability or otherwise of the risk managers to address the uncertainties; *independence* refers to the guarantee that risk assessors and managers are not influenced by the parochial interests of (powerful) stakeholders; and *responsiveness* refers to the timely and accurate sharing of information by risk managers (EFSA, 2012). These are important for building stakehohlder trust in risk managers and their work/decisions.

Risk communication does not focus only on risks. It also covers communicating benefits, to enable stakeholders make informed choices. An example from FAO/WHO (2015) illustrates this point. Fish consumption may be associated with exposure to methyl mercury (a chemical hazard). For this hazard, immunocompromised groups and pregnant women are more susceptible to its adverse health effects. Fish is also the chief source of docosahexaenoic (DHA) acid and eicosapentaenoic acid (EPA), which are important omega-3 fatty acids with several health benefits (as earlier discussed). Therefore, for this hazard-food pair, risk communication must consider both the potential adverse effects of methyl mercury exposure and the nutritional/health benefits of DHA/EPA in fish. The benefits and the risks will vary for different segments of the population. Communicating only the risk of methyl mercury exposure in fish may lead to less intake of fish products by all segments of the population, thereby depriving non-vulnerable consumers the benefits of DHA.EPA intake. Furthermore, if a pregnant women, due to her vulnerability, stops intake of all fish products on the basis of a communication of only the risk, she will deprive the foetus a vital supply of DHA/EPA. Hence, risk communication must share both the risks and benefits, making clear how different sub-populations may be affected differently.

A related point to the risk/benefit communication is the need to identify and address risk perceptions, especially among consumers. Whereas some consumers may perceive otherwise high risks as low due, for example, to the absence of obvious harm (e.g. absence of immediate adverse effect from exposure to carcinogenic chemical hazards), others may perceive low risks of hazard *A* as high due to antecedent experience of an acute adverse health effect of hazard *B*. Ultimately, it is risk perception that drives consumer attitudes and practices (FAO/WHO, 2015), hence it is vital in risk communication to effectively address any perceptions that do not accord with available scientific evidence.

An important requirement in risk communication is tailoring the communication strategy to the need on the ground. In emergency situations (such as a foodborne disease outbreak or an urgent product recall), direct, frequent messages are used for the communication, ensuring proper coordination to avoid confusing the public with conflicting information (FAO/WHO, 2015; EFSA, 2012). For an enduring food safety problem, such as food-handling-related contamination, a sustained communication with refined messages is recommended (FAO/WHO, 2015).

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Given the significant part smoked fish occupies in the Ghanaian diet, it is important to clearly identify which hazards in the commodity should be prioritized for food safety management. That was the focus of the first objective of this study, and is addressed in the next chapter.

CHAPTER 2: SCREENING SELECTED FOOD SAFETY HAZARDS IN TRADITIONALLY SMOKED FISH IN GHANA FOR THEIR RELEVANCE TO PUBLIC HEALTH

Redrafted from:

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ABSTRACT

The study sought to determine the most important food safety hazards linked to the processing technique of traditionally smoked fish in Ghana. It assessed the occurrence (prevalence and concentration) of selected microbiological contaminants (*Salmonella* sp., *Escherichia coli* and *Pseudomonas* sp.), biogenic amines (β-phenylethylamine, putrescine, cadaverine, histamine and tyramine), and polycyclic aromatic hydrocarbons (PAHs) (benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) in traditionally smoked fish sampled from selected markets and a processing site in Ghana, and screened them for their relevance (significance to public health) using a hazard screening decision flowchart. Eighty (80) samples of smoked *Sardinella* sp. and *Sphyraena* sp. collected from markets (n=20 per species) and a processing site (n=20 per species) were analysed for the aforementioned hazards. For each group of hazards, a representative hazard was selected based on regulatory focus as follows: *Salmonella* sp. for microbiological hazards, histamine for biogenic amines and benzo(a)pyrene for PAHs. The representative hazards were then screened for relevance using the flowchart. A proof of concept was also developed for screening the hazards for relevance based on their concentrations, prevalence and regulatory limits. Three categories were set: high relevance (hazard score (Hs) = 6-9), medium relevance (Hs = 3-4) and low relevance (Hs = 1-2). *Salmonella* sp. was not detected in any of the 80 samples. Histamine and benzo(a)pyrene both recorded 100% prevalence at concentrations ranging from <10 to 48 ± 7 mg/kg and 11 ± 2 to 75 ± 19 µg/kg, respectively. The highest histamine level was four-times lower than the EU maximum limit, whereas benzo(a)pyrene levels exceeded the EU maximum limit by up to 38-times. From the use of the decision flowchart, PAHs emerged as relevant hazards in smoked fish, whiles biogenic amines and microbiological hazards did not. Similarly, the hazard screening proof of concept also flagged PAHs to be of high relevance (Hs = 9), biogenic amines to be of medium relevance (Hs = 4) and microbiological hazards to be of low relevance (Hs = 1). The findings highlight PAHs as the most important hazards requiring attention in smoked fish in Ghana.

Keywords: Hazard identification, risk ranking, smoked fish

2.0 INTRODUCTION

In light of the domestic food security importance of smoked fish in Ghana (discussed in Chapter 1), the management of the occurrence of food safety hazards in the commodity and their associated risks to human health is an important task for regulators and a justified concern for consumers. At the international level, smoked fish food safety lapses such as listings on the EU rapid alert system for food and feed (RASFF) pose a reputational risk that may limit market access and thus hamper export revenue generation not only from the product concerned, but from food exports in general (Mindjimba et al., 2019).

Calls have been made for low- and middle-income countries (LMICs) to shift from focusing concerted food safety governance solely on foods for exports to including foods for domestic consumption (Unnevehr, 2014; Grace, 2015). In response to this, decisions made on the premise that all hazards are important may put undue pressure on the limited resources in such economies (Langerholc et al., 2018). It is necessary to first evaluate the hazard-food combinations that are relevant in food value chains within particular national and cultural contexts, in order to inform the nature of, and the implementation schedule for, domestic food safety governance (Grace, 2015). For such purposes, the public health significance of hazard-food combinations are determined through methods such as risk ranking and risk assessment (see Chapter 1). These provide a transparent, scientifically sound basis for risk management and risk communication (Langerholc et al., 2018).

As highlighted in Chapter 1, an important first step in risk ranking and risk assessment is the identification of relevant hazards in the food of interest in the context. According to FAO (2020), a relevant hazard is one that is potentially a source of risk to public health in a given food within a given context. Although multiple hazards may be detected in one food, not all of them may have a significant impact on public health as far as that food is concerned. Therefore, screening hazards for relevance seeks to focus attention on hazards that are important to the goal of protecting public health as far as the food under assessment is concerned. This study, therefore, sought to gather data for that first step of risk ranking and risk assessment by determining the occurrence of selected food safety hazards in traditionally smoked fish in Ghana, as influenced by the traditional processing method. The study determined the prevalence and concentration of selected microbiological and chemical hazards in traditionally smoked fish in Ghana and screened them for their relevance for food safety (risk) management within the Ghanaian context. The hazards considered were: microbiological contaminants (*Salmonella* sp. as food safety indicator, *Escherichia coli* as hygiene indicator, *Pseudomonas* sp. as indicator for biogenic amine producers); biogenic amines (β-phenylethylamine, putrescine, cadaverine, histamine and tyramine), and PAHs (benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene). The selection of these hazard groups was informed by literature on the (potential) association of the named hazards with the traditional fish smoking method (Chapter 1). For the hazard screening for relevance, a recent decision flowchart suggested by FAO (2020) was applied.

The study also proposes a proof of concept for a matrix-based scoring method that may be applied for screening hazards for relevance in situations where quantitative data on the concentration and prevalence of hazards are available. Food safety regulatory limits are set with a view to protecting public health (Laky, 2019) such that hazard levels below the limits are considered not to raise concerns for public health, while those above the limits do. Given that domestic food safety regulatory limits consider the characteristic food production, processing, preparation, consumption habits and their associated hazards, a matrix-based hazard scoring approach was developed around this fact as a proof of concept to support the screening of food safety hazards for their relevance. Such a scoring matrix may potentially find application for hazard relevance screening in situations where actual hazard concentration and prevalence data are available while epidemiological data specifically implicating a particular hazard-food combination in foodborne illnesses in that context is unavailable.

2.1 MATERIALS AND METHODS

An overview of the study methodology is provided in Fig. 2.1. Briefly, hazards potentially linked to traditionally smoked fish due to the processing technique and handling practices were identified by literature review (Chapter 1). Smoked-soft and smoked-dry fish (species specified hereafter) were sampled from selected markets in Accra (Ghana's capital) and also directly from processing sites and analysed for the concentrations and prevalence of the hazards of interest. The hazards were then screened for their relevance using a hazard screening decision flowchart suggested by FAO (2020). A proof of concept for hazard screening based on a comparison of hazard occurrence data (concentration and prevalence) to regulatory limits was also proposed.

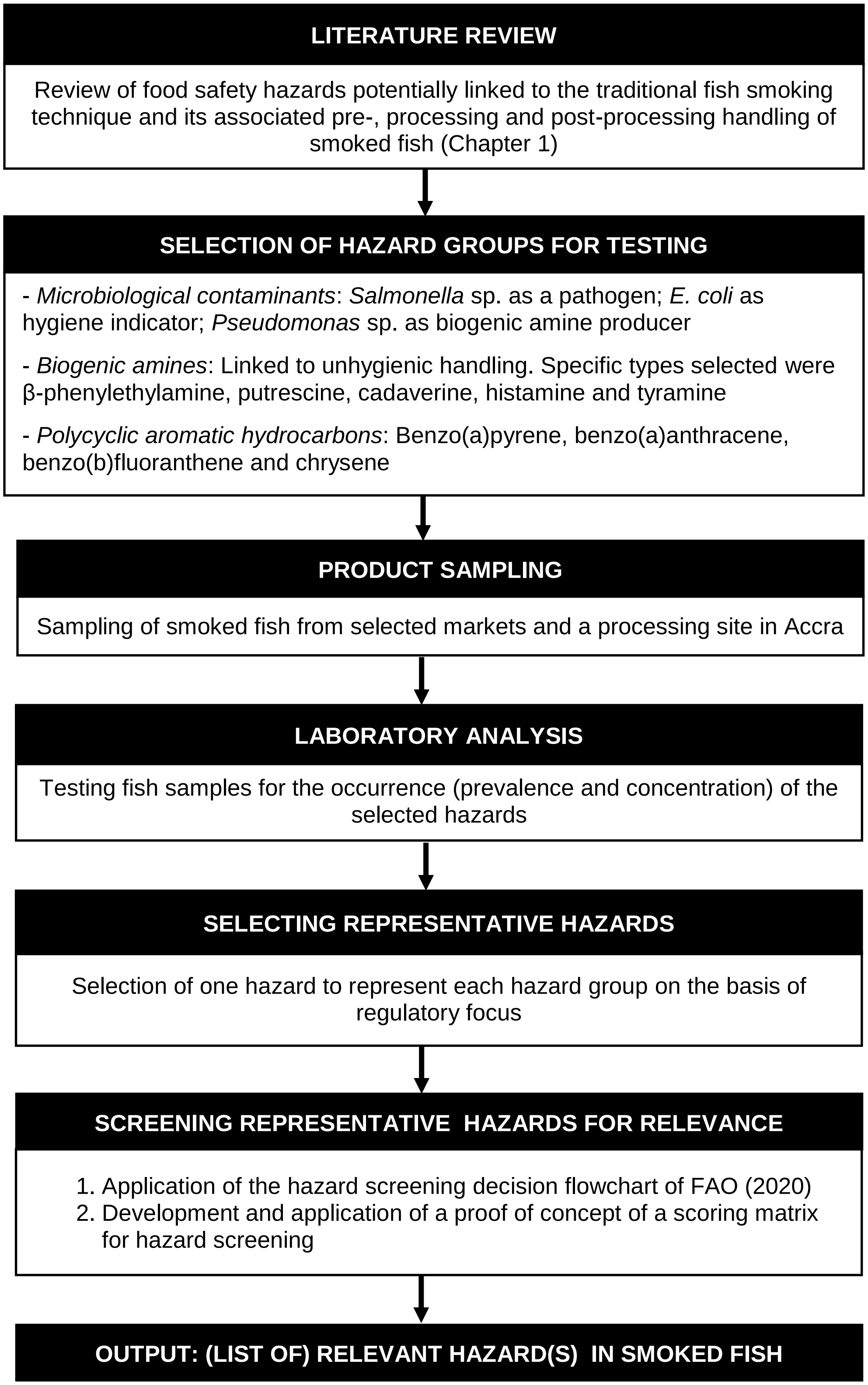

Fig. 2.1: Scheme used for the screening of food safety hazards in traditionally smoked fish

2.1.1 Study area, fish species and product types

The study was conducted in Accra, the capital of Ghana, having peri-urban communities known for their harvesting, processing and trading of fish (Atta-Mills et al., 2004). Two major markets (namely the Adabraka Fish Market and the Madina market) and a prominent fish processing site in Tema New Town were selected as sampling areas. The Adabraka Fish Market was selected because it is a dedicated sales point for traditionally processed fish in Accra. The Madina market is one of the main markets for food and non-food commodities in Accra and was selected by convenience (proximity to the University of Ghana where preliminary sample preparations and storage were done).

The fish species of interest were *Sardinella* sp., considered the most available and consumed fish species in Ghana (Essumang et al., 2012) and *Sphyraena* sp., a less available species. The two product forms of smoked-fish were evaluated (i.e. smoked-soft and smoked-dry, described in Chapter 1, Section 1.3).

2.1.2 Smoked fish sampling

Smoked fish products were sampled from markets and a processing site to ascertain differences in hazard levels due to post-processing contamination. The market samples and processing site samples were ***not*** related; that is, in this study, it was not the same products from the processing sites that were sent to the markets. At the markets, the products were purchased as normally sold to consumers. A total of 40 samples were purchased (2 fish species x 2 product forms x 2 markets x 5 sampling rounds = 40 samples). At each sampling round, approximately 5kg of each smoked product was purchased. For the processing site samples, frozen *Sardinella* sp. and *Sphyraena* sp. were purchased from cold stores and processed into smoked-soft and smoked-dry products on the Chorkor smoker and metal drum oven, according to the traditional fish smoking process flow described in Chapter 1 (Section 1.3.3.2). Smoking sessions were conducted by a career fish processor, in five replicates (2 fish species x 2 products x 2 ovens x 5 replicates = 40 samples). For each smoking session, approximately 18kg of fish was smoked. For each experimental replicate, raw (frozen) fish were also sampled immediately after opening the cartons of fish from the cold store. These raw samples were not handled by the processors.

2.1.3 Laboratory analysis

2.1.3.1 Sample preparation for chemical analysis

Ten fishes from each sampling point were homogenized as-is with a Waring® CB15V Heavy Duty Commercial blender (Waring Commercial, Stamford, CT, USA). Homogenates were vacuum-packed with a Henkelman® JUMBO 42 vacuum packaging machine (Henkelman USA, Elmhurst, IL, USA) and kept frozen at -22ºC until analysed.

2.1.3.2 Microbiological evaluation

Samples for microbiological tests were transported to the laboratory on ice and under aseptic conditions for immediate analysis (ten pooled fishes per sample). The reference testing methods were NMKL 125(2005) for the enumeration of *Escherichia coli*, ISO 13720: 2010 for the enumeration of *Pseudomonas* sp., and NMKL 71(1999) for the detection of *Salmonella* sp. in 25 g sample.

2.1.4 Determination of biogenic amines

Biogenic amines were determined using an adjusted Ultra High Performance Liquid Chromatography (UHPLC) protocol with derivatization by dansyl chloride, based on the method described by Komprda et al., (2009). Briefly, the fish proteins in 4g homogenized samples were precipitated with trichloroacetic acid, the extract filtered and the pH adjusted to 9.5 for derivatisation. Derivates were extracted from the aqueous phase with dichloromethane and evaporated under nitrogen to obtain a stable dry extract. The dry extract was dissolved in acetonitrile/water (65/30 ratio v:v) and filtered with 0.45 µm filters into HPLC-vials. The biogenic amine-derivates were separated by a gradient elution with H_2O/acetonitrile (time 0-23 min: H_2O 35-0%, acetonitrile 65-100%) on a Dionex bonded silica Acclaim TM RSLC 120 C18 reverse phase column (2.1 mm x 150 mm, particle size 2.2 µm) at a flow rate of 0.5 mL/min using a DAD detector at 225 nm. Limit of quantitation (LOQ) was 10 mg/kg.

2.1.5 Determination of PAH level

The analyses of PAH were carried out according to an ISO 17025 accredited method in a commercial laboratory (SGS, Hamburg, Germany). The samples (already homogenized, vacuum packed and then frozen) were shipped from Ghana to Belgium by air, on dry ice, for the analysis. For the testing, 2 g of homogenized fish was treated with 20 mL of hexane and 10μL of IS solution (a mix of isotopic labelled PAH all at 1 μg/mL: benzo[a]anthracene-d12, chrysene-d12, benzo[b]fluoranthene-d12, benzo[k]fluoranthene-d12, benzo[a]pyrene-d12, indeno[1,2,3-c,d]pyrene-d12, dibenzo[a,h]anthracene-d14 and benzo[g,h,i]perylene-d12, dibenzo[a,i]pyrene-d14). After shaking, the mixture was held for 1h in an ultrasound bath and then placed in the freezer for 2 h at −20^{o}C. Frozen fat and solid components were separated by centrifuging for 2 min at 5000 rpm, RCF 3,857 (Hettich Universal 320; Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The clear supernatant was transferred to a 10 mL vial and placed into a tray of a Gerstel MPS 2XL Sampler (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). An in-house and property owned clean-up method was employed using 0.25 g sodium sulphate in an MPS sample tray fitted on a BEKOlut SPE cartridge (BEKOlut GmbH & Co. KG, Hauptstuhl, Germany). After clean up, samples were immediately injected in a gas chromatograph-mass spectrometer. The gas-chromatograph (model 7890B; Agilent Technologies Inc., Santa Clara, CA, USA) consisted of a programmed temperature vaporization injector (50^{o}C/min, 500^{o}C/min to 320^{o}C; purge 1 min) and a Model 5977B mass spectrometer (Agilent Technologies Inc.). The column was a J&W DB 35MS (Agilent Technologies Inc.) (30 m × 0.25 mm inner diameter, 0.25 μm film) in an oven with the temperature programmed as: 50^{o}C, 3 min isotherm, 30^{o}C/min to 200^{o}C, 4^{o}C/min to 300^{o}C, 19 min isotherm. The carrier gas was helium at a constant flow of 1.0 mL/min. The injection volume was 100 μL. The quantifier and qualifier ions were: 252.1 and 250.0 for benzo[a]pyrene; 228.1, 226.1 and 229.0 for benzo[a]anthracene; 228.1, 226.1 and 229.0 for chrysene; and 252.1 and 250.0 for benzo[b]fluoranthene. The method was calibrated in the range 0.2 – 10 ppb (r^2 = 0.999). When higher concentrations were retrieved, the analysis was repeated with diluted samples. Relative standard deviations were typically 2.6% in standard solutions at 5 ppb. The limit of quantitation was 0.20 μg/kg.

2.1.6 Determination of hazard relevance

A two-step approach was used to arrive at the relevant hazards within the groups considered (see Fig. 2.1). Firstly, within each group of hazards (i.e. microbiological hazards, biogenic amines and PAHs), those used as markers in food safety regulations were selected to represent their group. Secondly, the representative hazards were screened for relevance using a decision flowchart developed by FAO (2020) for that purpose.

2.1.6.1 Selection of representative hazards

Salmonella sp., histamine and benzo(a)pyrene were selected as representative hazards for microbiological hazards, biogenic amines and PAHs, respectively. *Salmonella* sp. was selected as the pathogen among the organisms of interest (CDC, 2016). Histamine is considered by the Codex as an indicator for biogenic amines and as the most significant agent for scombrotoxin fish poisoning (SFP) (CAC, 2012), hence its selection. Benzo(a)pyrene is also a recognized marker for PAHs in foods (JECFA, 2005; EFSA, 2005).

2.1.6.2 Screening representative hazards for relevance

The decision flowchart adopted for screening each of the representative hazards is shown in Fig. 2.2. In applying the flowchart, node 4 was modified as "*Is foodborne exposure a **potentially** significant source of illness in the country?*" since epidemiological/surveillance data specifically linking foodborne illness from the representative hazards in smoked fish in Ghana were not found.

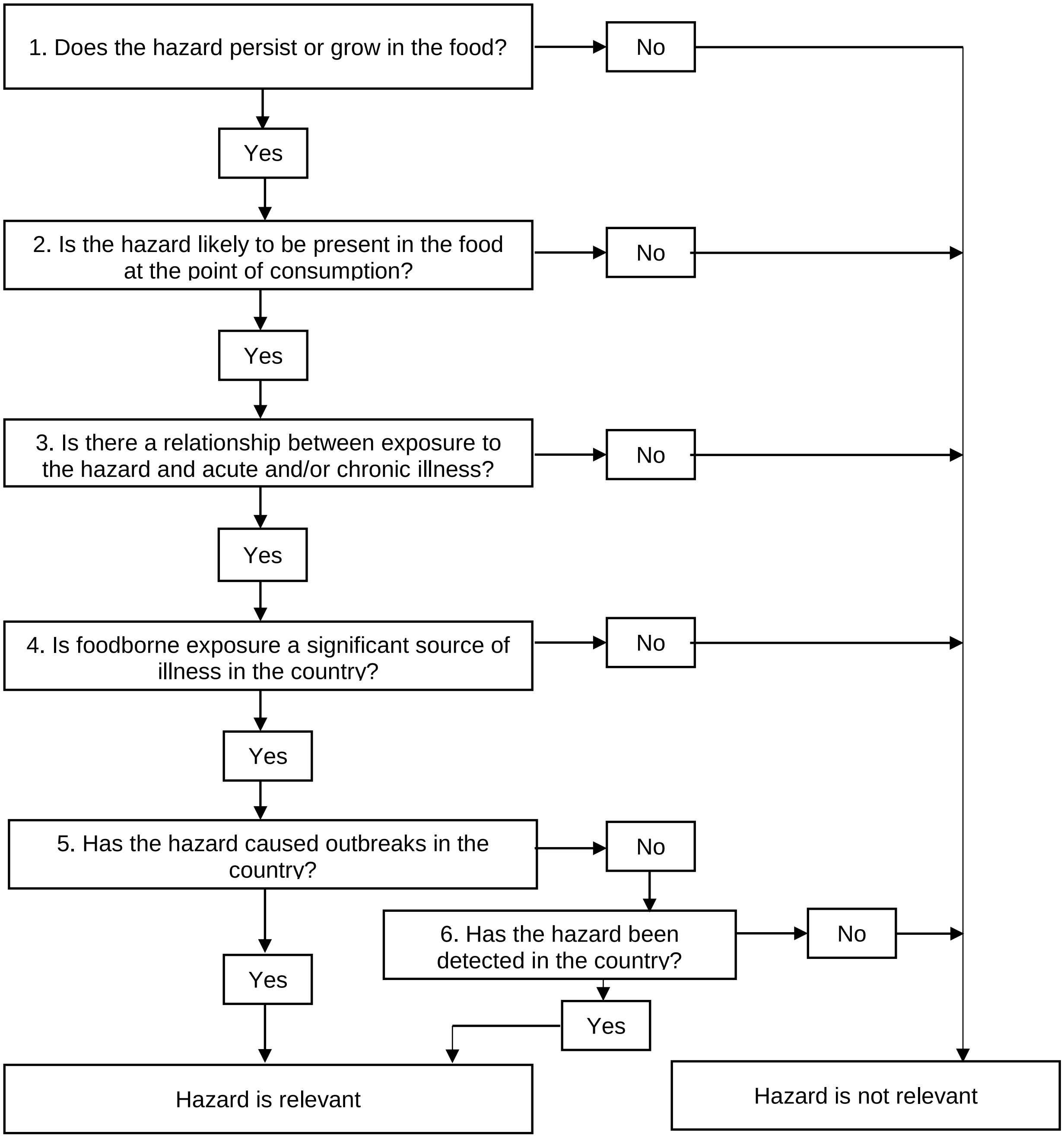

Fig. 2.2: Decision flowchart for screening food safety hazards for their relevance (FAO, 2020). Node 4 was modified as "*Is foodborne exposure a potentially significant source of illness in the country*?" due to lack of epidemiological/surveillance data specifically linking foodborne illness from the representative hazards in smoked fish in Ghana

2.1.7 Development of a hazard scoring matrix as a proof of concept for screening food safety hazards for relevance based on concentration and prevalence data

The proposed matrix assigns scores to hazards (called hazard scores, Hs) as a function of the concentration and prevalence of the hazards, as illustrated in Fig. 2.3. The two parameters were chosen since the concentration shows the extent to which the hazard level exceeds or falls below the regulatory limit, while the prevalence shows how pervasive is the occurrence of contamination in the smoked fish. Both are important for identifying appropriate measures for improving the safety of the food product.

To assign an Hs to a hazard, first, a concentration score (Cs) is assigned to the hazard by comparing of the hazard level in the product to its regulatory limit and analytical limit of quantitation (LOQ). The Cs scores proposed are 1 (lowest score, for when the concentration is less than the LOQ), 2 (medium score, for when the concentration is higher than the LOQ but lower than the regulatory limit) and 3 (highest score, for when the concentration exceeds the regulatory limit) (Fig. 2.3). Secondly, a prevalence score (Ps) is assigned as 1 (lowest score, for prevalence below 10%), 2 (for prevalence between 10 and 40%) and 3 (for prevalence above 40%.) Finally, an Hs is computed as a product of Cs and Ps, and interpreted according the matrix in Fig. 2.3.

Step 1: Assign a concentration score (Cs) to each hazard

Condition	Cs
C < LOQ	1
LOQ ≤ C ≤ Regulatory limit	2
C > Regulatory limit	3

C = Concentration of hazard in the product
LOQ = Limit of quantitation

Step 2: Assign a prevalence score (Ps) to each hazard

Condition	Ps
P < 10%	1
10%≤ P ≤ 40%	2
P >40%	3

P = Prevalence of hazard in the product

Step 3: Calculate and interpret a hazard score (Hs) for each hazard

Hs = Cs x Ps

		Cs			
		1	2	3	
Ps	1	1	2	3	**Hs = (Cs x Ps)**
	2	2	4	**6**	
	3	3	**6**	**9**	

Hazard score 6 – 9 = High relevance (Red);
Hazard score 3 – 4 = Medium relevance (Amber);
Hazard score 1 – 2 = Low relevance (Green)

Fig. 2.2: Scheme of a proof of concept for screening food safety hazards for relevance in particular domestic contexts

2.1.7.1 Application of the hazard score matrix to screen the representative hazards in smoked fish

In applying the proof of concept to the present study, for *Salmonella* sp., since a detection protocol was used, Cs were assigned as "absent" = 1; or "present" = 3. LOQ values were 10 mg/kg for histamine and 0.2 µg/kg for benzo(a)pyrene. The regulatory limits used were "absent in 25 g" for *Salmonella* sp. (Uyttendaele et al., 2018), 200 mg/kg for histamine (Commission Regulation (EC) No. 2073/2005) and 2 µg/kg for benzo(a)pyrene (Commission Regulation (EU) No. 835/2011).

The screening was done at two levels: product-specific and food-category hazard screening. The former was applied to each product type (smoked-soft and smoked-dry forms of both *Sardinella* sp. and *Sphyraena* sp.) at each sampling point. This served to show how, for the same product , relevant hazards may change with the point of the value chain considered.

For the food-category hazard screening, all the smoked fish products were considered as one food category (i.e. as "smoked fish", regardless of fish species, product type or sampling point). In this case, for each hazard, the mean Hs was calculated from the Hs values of each sampling point. This was done to give an overall picture of the relevance of each hazard in "smoked fish" as a food category.

2.1.8 Data analysis

Hazard concentrations are reported on as-is basis to reflect the normal conditions of food consumption. Concentration and prevalence data were analysed using the Statistical Package for the Social Sciences software (SPSS®, version 22). Tests for normality were done with the Shapiro-Wilk test. Means of normally distributed data were compared by independent t-tests and ANOVA, as appropriate. Where data were not normally distributed, non-parametric tests were used to compare means.

2.2 RESULTS AND DISCUSSION

Generally, the results show that, where detected, levels of the hazards were highest ($p<0.05$) in samples from the markets than in those collected directly from the processing site (Tables 2.1 to 2.3). None of the hazards was detected in the raw fish samples, suggesting that processing and handling accounted for the occurence in the smoked fish from the two sampling points considered (Tables 2.1 to 2.2). In the case of microbiological counts (*E. coli* and *Pseudomonas sp.*, Table 2.1) and biogenic amine levels (Table 2.2), unsanitary handling practices during and after processing may account for the observation (Morreno and Tores, 2001), since those organisms and the stated compounds are hygiene indicators (Silla Santos, 1996; Park et al., 2010; Hu et al., 2012). Other studies in Ghana (Coffie, 2002; Adu-Gyamfi, 2006) and neighbouring Nigeria (Ineyougha et al., 2015; Akinwumi and Adegbehinge, 2015) attributed poor microbial profile of smoked fish on markets to unsanitary conditions. For PAHs (Table 2.3), the smoking process is invariably the source of contamination at the processing site (Singh et al., 2016).

2.2.1 Occurrence of *Salmonella* sp., *Escherichia coli* and *Pseudomonas* sp.

The microbiological quality of the smoked products was expected to be influenced by the initial microbial load of the raw fishes, handling conditions during processing, the impact of the smoking treatment, and potential (re-)contamination post-processing. Since none of the organisms was detected in the raw material (frozen fish), any cell counts in the processing site samples should be attributed to processing and handling.

Salmonella sp. was not detected in any of the products (absent in 25g) (Table 2.1), corroborating findings of earlier studies in Ghana that also did not detect the pathogen in traditionally smoked fish (Nerquaye-Tetteh et al., 2002; Adu-Gyamfi, 2006; Amuna, 2014). In Benin, where the climate and fish smoking and handling practices are similar to the situation in Ghana, Anihouvi et al. (2018) screened raw and traditionally smoked fish for microbiological hazards and did not detect *Salmonella* sp. These suggest that the organism may not be a priority microbiological hazard for traditionally smoked fish.

From Table 2.1, although *E. coli* was present at levels up to 2.9 log CFU/g, a 6 log or higher reduction in cell numbers will be achieved with a 70ºC heat treatment for 2 minutes (Stringer et al., 2000; Osaili et al., 2007). A typical Ghanaian soup or stew, for which smoked fish is typically used, is cooked under more stringent time-temperature conditions (≥80ºC for ≥30minutes) (Bomfeh, 2011) and may thus be adequate to eliminate that microbial load. The same can be said for several vegetative microbiological pathogens such as *Salmonella* sp., should they be found in the products. Hence, at the consumer level in the Ghanaian context, these numbers may lose their food safety significance. Vegetative bacterial hazards may, therefore, be considered to have a low food safety significance for the commodity in that context. This, however, does not negate the need to ensure the application of good manufacturing and good hygienic practices during traditional fish smoking nor the need to improve hygienic conditions of post-processing handling, to limit the growth of (toxin-producing) pathogens.

Table 2.2: Microbiological status of traditionally smoked fish from markets and from a processing site in Accra

Fish species	Product	Sampling point	***E. coli*** **(mean log CFU/g ±stdev)**	***Pseudomonas*** **sp. (mean log CFU/g± stdev)**	***Salmonella*** **sp.**
Sardinella sp.	Raw fish*	Processing site (n=5)	ND	ND	Absent in 25g
	Smoked-soft	Chorkor smoker (n=5)	1 ± 0.[a]	1.3 ±0 .6[a]	Absent in 25g
		Metal drum oven (n=5)	1 ± 0[a]	1 ± 0[b]	Absent in 25g
		Market (n=10)	1.1 ± 0.2[a]	2.6 ± 0.3[c]	Absent in 25g
	Smoked-dry	Chorkor smoker (n=5)	1 ± 0[a]	1 ± 0[a]	Absent in 25g
		Metal drum oven (n=5)	1 ± 0[a]	1 ± 0[a]	Absent in 25g
		Market (n=10)	1.1 ± 0.1[a]	2.1 ± 0.1[b]	Absent in 25g
Sphyraena sp.	Raw fish*	Processing site (n=5)	ND	ND	Absent in 25g
	Smoked-soft	Chorkor smoker (n=5)	1.4 ± 0.2[a]	1.5 ± 0.4[a]	Absent in 25g
		Metal drum oven (n=5)	1. ± 0[b]	1 ± 0[b]	Absent in 25g
		Market (n=10)	2.9 ± 0.9[c]	3.8 ± 0.4[c]	Absent in 25g
	Smoked-dry	Chorkor smoker (n=5)	1 ± 0[a]	1.1 ± 0.1[a]	Absent in 25g
		Metal drum oven (n=5)	1 ± 0[a]	1 ± 0[a]	Absent in 25g
		Market (n=10)	2.6 ± 0.4[b]	2.5 ± 0.9[b]	Absent in 25g

For each product, counts in columns with different superscripts differ significantly ($p<0.05$)
*Frozen fish sampled directly and immediately from their cartons as purchased from cold stores
ND = not detected

2.2.2 Occurrence of biogenic amines

The biogenic amine levels in the products are reported in Table 2.2. Generally, compared to the EU limits of 200 mg/kg for histamine, contamination levels in the products were low, ranging from <10 mg/kg to 48 ± 7 mg/kg. As was observed for the microbial contaminants, occurrence of these compounds in the smoked products could be attributed to unhygienic handling prior to and after thermal processing, since the levels in the raw fish (which, as earlier mentioned, were not handled by the processors) were below the limit of quantitation (<10 mg/kg).

Biogenic amine levels in market samples were up to 5 times higher than in those from the processing site (Table 2.2). The highest *Pseudomonas* sp. counts (Table 2.1) also corresponded with the highest biogenic amine levels (Table 2.2). For example, for smoked-soft and smoked-dry *Sardinella* sp., respective counts of 2.6 log CFU/g and 2.1 log CFU/g of *Pseudomonas* sp. were the highest levels and occurred in market samples (Table 2.2). The corresponding histamine levels in the same products were 20 ± 8 mg/kg and 16 ± 7 mg/kg, respectively, also in market samples (Table 2.2). Therefore, for the market samples, improper handling of their corresponding raw fish prior to processing potentially resulted in contamination and proliferation with biogenic amine producers (beside *Pseudomonas* sp.) and may have contributed to the observation.

Among the biogenic amines, histamine is considered the most important for food safety due to its implication in foodborne illnesses (Leuschner et al., 2013; Lehane and Olley, 2000; JECFA, 2013). In Europe, histamine is the only biogenic amine for which a regulatory limit has been set, the value also being the Codex recommended limit of 200 mg/kg (Commission Regulation (EC) No. 2073/2005). From Table 2.2, although histamine was present in all the smoked fish products, the highest concentration was 48 ± 7 mg/kg (in smoked-dry *Sphyraena* sp. from the market), which is four times lower than the regulatory limit of 200 mg/kg, suggesting a low public health significance. Their occurrence in all the smoked products, however, points to the need to improve hygienic conditions of handling.

Table 2.2: Biogenic amine levels in traditionally hot-smoked fish from markets and from a processing site in Accra

Fish species	Product*	Sampling point	Biogenic amine level (mean±stdev mg/kg)				
			β-Phenylethylamine	**Putrescine**	**Cadaverine**	**Histamine**	**Tyramine**
Sardinella sp.	Raw fish	Raw fish (n=5)	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]
	Smoked-soft	Chorkor smoker (n=5)	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]
		Metal drum oven (n=5)	<10[a]	11 ± 2[b]	10 ± 0.4[b]	13 ± 3[b]	13 ± 3[b]
		Market (n=10)	<10[a]	23 ± 3[c]	67 ± 7[c]	20 ± 8[c]	22 ± 3[c]
	Smoked-dry	Chorkor smoker (n=5)	<10[a]	<10[a]	10 ± 0.2[a]	<10[a]	<10[a]
		Metal drum oven (n=5)	<10[a]	<10[a]	18 ± 0.4[b]	<10[a]	<10[a]
		Market (n=10)	<10[a]	16 ± 5[b]	59 ± 9[c]	16 ± 7[b]	11 ± 3[b]
Sphyraena sp.	Raw fish	Raw fish (n=5)	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]
	Smoked-soft	Chorkor smoker (n=5)	<10[a]	<10[a]	13 ± 2[b]	<10[a]	<10[a]
		Metal drum oven (n=5)	<10[a]	<10[a]	16 ± 7[b]	<10[a]	<10[a]
		Market (n=10)	<10[a]	<10[a]	42 ± 13[c]	<10[a]	<10[a]
	Smoked-dry	Chorkor smoker (n=5)	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]
		Metal drum oven (n=5)	<10[a]	<10[a]	<10[a]	<10[a]	<10[a]
		Market (n=10)	<10[a]	<10[a]	35 ± 14[b]	48 ± 7[b]	10 ± 5[b]

For each product, concentrations for sampling points with different superscripts are statistically significant ($p<0.05$)

*Oven samples are direct products from the raw fish. However, market samples are neither related to the raw fish nor oven samples.

2.2.3 Occurrence of polycyclic aromatic hydrocarbons (PAHs)

Unlike the other hazards discussed, PAHs were present in all the smoked fish products at concentrations exceeding the EU regulatory limits by up to 38 times (Table 2.3). Reference is made to EU limits since Codex has not yet set standards for PAHs in smoked fish (Ingenbleek et al., 2019), and the Ghana Standards Authority adopts the EU standards, chiefly for the regulation of smoked fish exports (GSA, 2014). PAH discussions are also focused on BaP and PAH4 since those are the regulatory markers for the hazards (EFSA, 2008).

Market samples were the most contaminated, exceeding the levels in the processing site samples by up to seven-times for BaP and up to 10-times for PAH4 (Table 2.3). These higher PAH levels in the market samples could be attributed to the practice of re-smoking, in which, to prevent insect infestation and mould growth, fish processors often subject unsold products and those in storage to heat and smoke (Ahadzi, 2017). The heat and smoke treatments used in this practice are less intense than applied during processing of raw fish (Ahadzi, 2017), but may nevertheless contribute to increasing PAH levels in the products.

The PAH data compare with reported levels of the same contaminant in smoked fish in African countries. As shown in Table 1.4 (Chapter 1), although studies in Nigeria, Uganda, Tanzania, Benin, Cameroun and Mali reported differing extents of PAH contamination in smoked fish, they were all in agreement on the point that the contaminant levels exceeded the EU maximum limits, as was found in the present study. It is noted that the operational principles underlying the fish smoking techniques in these countries are the same: fish is cooked over dry heat from burning fuelwood on unsophisticated ovens (Peñarubia et al., 2017). Additionally, handling practices such as re-smoking is common across the countries (Adeyeye and Oyewole, 2016).Therefore, along with the data of the present study, these reports highlight the traditional smoking technique as the chief cause of PAH contamination of smoked fish. Ingenbleek et al. (2019) also reported that among 16 core food commodities in Africa tested for PAHs, smoked fish were the most contaminated. The study found a 100% occurrence of the hazard at 100% exceedance of EU limits (ibid.). Other foods tested included cooking oils, tubers (yam and cassava), peanut, and spices.

Products from the metal drum oven had significantly lower ($p<0.05$) PAH levels than Chorkor smoker products (Table 2.3). This could be attributed to the higher degree of smoke retention in the latter oven. Longer exposure to smoke has been cited as a contributor to high PAH levels in smoked products (CAC, 2009). As shown in Fig. 1.2 in Chapter 1, the practice of stacking several racks of fish and covering the topmost rack when using the Chorkor smoker serves to concentrate smoke around the fish. This is not observed in the use of metal drum oven (Figs. 1.2). The characteristic of better smoke retention in the Chorkor smoker was considered an advantage when that oven was developed in Ghana in 1969, since it allowed better flavouring of products (Brownell, 1983; Nerquaye-Tetteh et al., 2002). It is seen, however, that this apparent gain in sensory appeal led to a further compromise of the safety of the products. Additionally, the higher capacity of the Chorkor smoker translates into a higher quantity of fish fat that could drip onto the heat source. This way, PAH generation by pyrolysis is enhanced (Rengarajan et al., 2015), thus potentially increasing the contamination level.

Concerning the impact of product type, smoked-dry products had up to two- and 13-times higher BaP and PAH4 levels, respectively, than smoked-soft products (Table 2.3). This may be due to the longer exposure of smoked-dry products to direct heat and smoke during processing (Ghele, 2009).

Table 2.3: PAH levels (mean ± stdev µg/kg) in smoked *Sardinella* sp. and *Sphyraena* sp. from markets and from a processing site in Accra

Product	Sampling Point	BaP	Benzo(a)anthracene	Benzo(b)fluoranthene	Chrysene	PAH4
Raw fish	Cold store (n=5)	ND	ND	ND	ND	ND
Smoked-soft *Sardinella* sp.	Chorkor smoker (n=5)	26 ± 5.[a]	45 ± 3	28 ±5	67 ± 3	167 ± 6[a]
	Metal drum oven (n=5)	11 ±2 [b]	13 ± 2	21 ± 3	13 ± 2	58 ± 4[b]
	Market (n=10)	48 ± 13[c]	135 ± 33	63 ± 11	165 ± 23	411 ± 66[c]
Smoked-dry *Sardinella* sp.	Chorkor smoker (n=5)	60 ± 4[a]	115 ± 8	72 ± 5	147 ± 10	395 ± 17[a]
	Metal drum oven (n=5)	25 ± 2[b]	37 ± 3	25 ± 2	48 ± 4	136 ± 11[b]
	Market (n=10)	63 ±14 [c]	149 ± 69	78 ± 24	179 ± 75	471 ± 18[c]
Smoked-soft *Sphyraena* sp.	Chorkor smoker (n=5)	50 ± 1[a]	75 ± 4	51 ± 3	94 ± 3	270 ± 10[a]
	Metal drum oven (n=5)	37 ± 7[b]	51 ± 10	35 ± 6	44.2 ± 0.1	168 ± 33[b]
	Market (n=10)	75 ± 19[c]	172 ± 4	84 ± 26	204 ± 63	534 ± 147[c]
Smoked-dry *Sphyraena* sp.	Chorkor smoker (n=5)	61 ± 6[a]	105 ± 6	72 ± 8	123 ±11	360 ± 29[a]
	Metal drum oven (n=5)	70 ± 4[b]	91 ± 28	63 ± 4	103 ± 7	327 ± 34[b]
	Market (n=10)	45 ± 24[c]	179 ± 49	103 ± 94	247 ± 95	564 ± 164[c]

For each product, BaP and PAH4 values with different superscripts differ significantly ($p<0.05$) among the sampling pointsr

2.2.4 Screening for hazard relevance using the decision flowchart

The outputs of the hazard relevance screening from the decision flowchart are summarized in Table 2.4. Among the three representative hazards, only BaP emerged as a relevant hazard in the smoked fish products. *Salmonella* sp. was eliminated at node 1, since it was not detected in any of the samples in this study, nor in previous studies (Nerquaye-Tetteh et al., 2002; Adu-Gyamfi, 2006; Amuna, 2014; Anihouvi et al., 2018). Histamine was eliminated at node 4, since it was not considered to be a potentially significant source of scrombrotoxin food poisoning in smoked fish in Ghana, considering that the highest contamination levels were four times lower than the EU maximum limit of 200 mg/kg (Commission Regulation (EC) No. 2073/2005). BaP was confirmed at node 6 as a relevant hazard in smoked fish products, due to its prevalence (100% in all products) and concentrations reaching 38-fold in excess of the maximum limit of 2 µg/kg (Commission Regulation (EU) No. 835/2011).

The decision flowchart outputs suggest that, for public health significance, PAHs are the relevant hazards linked to the traditional fish smoking method and its associated product handling.

Table 2.4: Outcome of screening benzo(a)pyrene, histamine and *Salmonella* sp. for relevance as food safety hazards in traditionally smoked fish products in Ghana, using the decision flowchart of FAO (2020)

	Hazard relevance		
Product	**Benzo(a)pyrene**	**Histamine**	***Salmonella* sp.**
Smoked-soft *Sardinella* sp.	Relevant Confirmed at node 6	Not relevant Eliminated at node 4	Not relevant Eliminated at node 1
Smoked-dry *Sardinella* sp.	Relevant Confirmed at node 6	Not relevant Eliminated at node 4	Not relevant Eliminated at node 1
Smoked-soft *Sphyraena* sp.	Relevant Confirmed at node 6	Not relevant Eliminated at node 4	Not relevant Eliminated at node 1
Smoked-dry *Sphyraena* sp.	Relevant Confirmed at node 6	Not relevant Eliminated at node 4	Not relevant Eliminated at node 1

2.2.5 Application of the hazard scoring proof of concept to screen hazards for relevance

The calculated hazard scores (Hs) for the representative hazards are presented in Table 2.5. BaP was the only hazard for which the maximum Hs value (9) was obtained, and that for all products and sampling points. The least Hs value (1) was obtained for *Salmonella* sp. across all products and sampling points. Thus, BaP was assigned a high relevance and *Salmonella* sp. a low relevance in all product types from all sampling points (Table 2.6). Histamine oscillated between high relevance (Hs = 6) and low relevance (Hs = 3), depending on the sampling point. In three out of the four cases where histamine was assigned a high relevance, the products were from the market (Table 2.6).

The results of the proposed hazard scoring approach are similar to the output of the decision flowchart as far as PAHs are concerned. Both approaches show that PAHs are relevant hazards in traditionally smoked fish in Ghana. However, some important differences are noted for the other hazards. Whereas with the flowchart *Salmonella* sp. and histamine were eliminated outright as not being relevant, with the hazard scoring, these hazards were not eliminated but rather assigned lower relevance. This may be considered an advantage of the hazard scoring approach since a complete elimination of a hazard as irrelevant (as occurs with the flowchart) may lead to neglect of otherwise important food safety concerns. For example, the occurrence of biogenic amines in all market samples (100% prevalence) clearly points to a lapse in hygienic handling conditions (both pre- and post-processing), although the concentrations were below regulatory limits. This is not picked up by the decision flowchart, whiles the proposed hazard scoring method flags the issue by assigning a medium to high relevance to histamine (Table 2.6). Thus, the hazard scoring captures nuances in hazard occurrence that could enable a more comprehensive food safety management.

Table 2.5: Prevalence scores, concentration scores and their corresponding hazard scores for representative food safety hazards in smoked fish in Ghana

		Salmonella sp.					BaP					Histamine				
		Prevalence		Concentration		Hazard Score	Prevalence		Concentration		Hazard Score	Prevalence		Concentration		Hazard Score
Product	Sampling Point	%	Ps	(Present/ Absent)	Cs	Cs x Ps	%	Ps	Mean (µg/kg)	Cs	Cs x Ps	%	Ps	Mean (mg/kg)	Cs	Cs x Ps
Smoked-soft *Sardinella* sp.	Market (n=10)	0	1	Absent	1	1	100	3	48 ± 13	3	9	100	3	20 ± 9	2	6
	Chorkor smoker (n=5)	0	1	Absent	1	1	100	3	26 ± 5.	3	9	100	3	<10	1	3
	Metal drum oven (n=5)	0	1	Absent	1	1	100	3	11 ± 2	3	9	100	3	13 ± 3	2	6
Smoked-dry *Sardinella* sp.	Market (n=10)	0	1	Absent	1	1	100	3	63 ± 14	3	9	100	3	16 ± 7	2	6
	Chorkor smoker (n=5)	0	1	Absent	1	1	100	3	60 ± 4	3	9	100	3	<10	1	3
	Metal drum oven (n=5)	0	1	Absent	1	1	100	3	26 ± 2	3	9	100	3	<10	1	3
Smoked-soft *Sphyraena* sp.	Market (n=10)	0	1	Absent	1	1	100	3	75 ± 19	3	9	100	3	<10	1	3
	Chorkor smoker (n=5)	0	1	Absent	1	1	100	3	50 ± 1	3	9	100	3	<10	1	3
	Metal drum oven (n=5)	0	1	Absent	1	1	100	3	37 ± 7	3	9	100	3	<10	1	3
Smoked-dry *Sphyraena* sp.	Market (n=10)	0	1	Absent	1	1	100	3	45 ± 24	3	9	100	3	48 ± 7	2	6
	Chorkor smoker (n=5)	0	1	Absent	1	1	100	3	61 ± 6	3	9	100	3	<10	1	3
	Metal drum oven (n=5)	0	1	Absent	1	1	100	3	70 ± 4	3	9	100	3	<10	1	3
				Mean Hazard Score		**1**			**Mean Hazard Score**		**9**			**Mean Hazard Score**		**4**

Ps = Prevalence score; Cs = Concentration score

Scoring criteria

Representative hazards: Microbial hazards - *Salmonella* sp.; biogenic amines - histamine; PAH – benzo(a)pyrene

Prevalence scores (Ps): if P <10%, Ps = 1; if 10% ≤ P ≤ 40%, Ps=2; if P >40%, Ps = 3, where P = prevalence

Concentration scores (Cs) for BaP and Histamine: if C < LOQ, Cs = 1; if LOQ ≤ C ≤ Regulatory limit, Cs = 2; if C > Regulatory limit, Cs = 3, where C = Concentration, LOQ = limit of quantitation

Concentration (C) scores for *Salmonella* sp.: Absent = 1; Present = 3

LOQ: Histamine = 10 mg/kg; Benzo(a)pyrene = 0.2 µg/kg

Regulatory limits: *Salmonella* sp.: Absent in 25 g; Histamine: 200 mg/kg; Benzo(a)pyrene: 2 µg/kg

Hazard score, Hs = Ps x Cs

Table 2.6: Hazard relevance in individual smoked fish products and in all smoked fish products considered as a food category

Product	Source	Hazard relevance [based on hazard scores (Hs)]		
		High relevance	**Medium Relevance**	**Low relevance**
Smoked-soft *Sardinella* sp.	Market	BaP (Hs = 9) Histamine (Hs = 6)	-	*Salmonella* sp. (Hs = 1)
	Chorkor smoker	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
	Metal drum oven	BaP (Hs = 9) Histamine (Hs = 6)	-	*Salmonella* sp.
Smoked-dry *Sardinella* sp.	Market	BaP (Hs = 9) Histamine (Hs = 6)	-	*Salmonella* sp. (Hs = 1)
	Chorkor smoker	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
	Metal drum oven	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
Smoked-soft *Sphyraena* sp.	Market	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
	Chorkor smoker	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
	Metal drum oven	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
Smoked-dry *Sphyraena* sp.	Market	BaP (Hs = 9) Histamine (Hs = 6)	-	*Salmonella* sp. (Hs = 1)
	Chorkor smoker	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
	Metal drum oven	BaP (Hs = 9)	Histamine (Hs = 3)	*Salmonella* sp. (Hs = 1)
All products as one food Category*	All sampling	BaP (Hs = 9)	Histamine (Hs = 4)	*Salmonella* sp. (Hs = 1)

*Hs calculated as the mean of the Hs values at all sampling points for all products. It allows screening of hazards for relevance across all products as one food category (i.e. traditionally smoked fish)

Another advantage of the proposed hazard scoring approach is that it allows determination of changes in relevance of the same hazard along the value chain, since it uses actual concentration and prevalence data linked to the sampling point. From Tables 2.2 to 2.4, it is seen that the concentration of the hazards differed between the sampling points, being higher at the markets than at the processing sites. These differences, for example, reflected in the hazard relevance for histamine at these points (Table 2.3 and 2.6). This way, the scoring approach can enable identification of points along the value chain where food safety concerns that are determinable by hazard concentrations and prevalence are present.

A challenge identified with the scoring approach is that it cannot be applied in situations where hazard concentration and prevalence data are not available. Note, however, that even for the flowchart, some analytical data is required (i.e. detection of the hazard in the context, see node 6 of Fig. 2.1), although the data demands are much less. Furthermore, the validity of the hazard scores depend heavily on the reliability of the concentration and prevalence data (e.g. as influenced by the sample size, sampling method, and reliability of laboratory analytical procedures). In this study, for example, a larger sample size than the 80 collected may have enhanced the generalizability of the results (Zwietering et al., 2016).

It should be noted that the proposed hazard scoring approach is not being pitted against the decision flowchart to determine which is a better global approach for screening hazards for relevance. Rather, the former is being presented as a proof of concept that may be used in situations where actual hazard concentration and prevalence data are available, as it will enable not only the selection of relevant hazards based on data from that context, but also highlight other hazards which in themselves may not have been flagged as relevant by the flowchart but which, nonetheless, may point to food safety challenges such as unhygienic post-processing handling.

2.2.6 Value of the hazard relevance screening

In principle, the hazard relevance screening (whether done with the decision flowchart or with the proposed hazard scoring matrix) only shows that a given hazard *by itself* has a public health significance. The screening does not show the differences in public health impact among hazards, and does not, therefore, show whether one hazard is *more* relevant than another. This is so because hazards may differ in their respective toxicological endpoints. Hence, to establish how different hazards that have been identified as relevant differ in their impact on public health, risk ranking needs to be performed (see Chapter 1). Risk ranking uses a homogenous basis (e.g. DALYs) to differentiate relevant hazards according to their public health impact (Van der Fels-Klerx et al., 2018; FAO, 2020). In fact, as earlier mentioned (see section 2.0), the screening of hazards for relevance is considered a preliminary step to risk ranking. In this study, since only one hazard emerged as relevant, a further risk ranking was not necessary. It may be argued that since the hazard scoring approach categorized the hazards as high, medium and low relevance, they could be subjected to risk ranking. However, the occurrence data vis-à-vis the regulatory limits clearly shows the ranking is not needed.

2.2.7 PAHs as the relevant hazards in smoked fish

The observation that PAHs are the relevant hazards in traditionally smoked fish (principally on account of the processing method and handling) among the contaminants considered corroborates findings in the first multi-centre sub-Saharan Africa Total Diet Study on PAHs (Ingenbleek et al., 2019), which found a 100% occurrence of the hazard in smoked fish, at a 100% exceedance of the EU regulatory limits. Smoked fish were also the most contaminated products with the hazard among the food groups tested (Ingenbleek et al., 2019).

EU border rejections of smoked fish from Ghana further highlight the extent of the problem. Table 2.7 shows a selection of notifications of the rapid alert system for food and feed (RASFF) for unacceptable PAHs in smoked fish from Ghana between 2013 and 2020. No such alerts were found for histamine and *Salmonella* sp. in the products. Considering that these products, which were expected to be produced in facilities regulated for compliance to the EU limits, failed the quality checks, the extent of the problem with smoked fish on the domestic market that are produced in traditional settings with the Chorkor smoker and the metal drum oven can be expected to be much higher, as seen from the study results.

Table 2.7: Some notifications of the EU rapid alert system for food and feed (RASFF) of unacceptably PAH levels in smoked fish entering the EU from Ghana (from 2013 – September 2020). EU Limit for Benzo(a)pyrene = 2µg/kg (Commission Regulation (EC) No. 835/2011)

Date	Country issuing notification	Notification details as provided on the RASFF query result
16-May-2013	Germany	Benzo(a)pyrene (61.2 µg/kg) in dried fish from Ghana, via Belgium
9-Aug-2013	Belgium	Benzo(a)pyrene (45.1 µg/kg) in smoked fish from Ghana
2-Jan-2014	United Kingdom	Benzo(a)pyrene (33 µg/kg) in smoked sardines (*Sardinella* sp.) from Ghana
3-Feb-2014	Belgium	Benzo(a)pyrene (36.7 µg/kg) and PAH4 (221 µg/kg) in smoked sardines (*Sardinella aurita*) from Ghana
6-Mar-2014	Germany	Benzo(a)pyrene (35 µg/kg) in smoked sardines (*Sardinella aurita*) from Ghana
26-May-2014	Belgium	Benzo(a)pyrene (6.2 µg/kg) and polycyclic aromatic hydrocarbons PAH4 (46.1 µg/kg) in smoked sardinellas (*Sardinella aurita*) from Ghana
04-Jul-2016	United Kingdom	Benzo(a)pyrene (80 µg/kg) and polycyclic aromatic hydrocarbons (sum PAH: 596 µg/kg) in salted smoked fish (*Lattes* sp.) from Ghana
27-Jul-2016	United Kingdom	Benzo(a)pyrene (27.6 µg/kg) and polycyclic aromatic hydrocarbons (PAH4 sum: 166.9 µg/kg) in smoked sardinellas from Ghana
16-Dec-2016	United Kingdom	Benzo(a)pyrene (81.2 µg/kg), polycyclic aromatic hydrocarbons (sum of PAH4: 507.9 µg/kg), benzo(a)anthracene (163.1 µg/kg), chrysene (198.4 µg/kg) and benzo(b)fluoranthene (65.2 µg/kg) in smoked catfish (*Clarias* sp.) from Ghana
13-Dec-2016	Germany	Benzo(a)pyrene (5.64 µg/kg) and polycyclic aromatic hydrocarbons (sum of PAH4: 60.92 µg/kg) in salted smoked sardines (*Sardinella* sp.) from Ghana
20-Nov-2019	United Kingdom	Benzo(a)pyrene (11.3 µg/kg) in smoked fish from Ghana
16-Mar-2020	United Kingdom	Benzo(a)pyrene (28.7 µg/kg) and PAH4 (266.1 µg/kg in smoked Sardinella sp. from Ghana

Source: Result of PAH alert search at https://webgate.ec.europa.eu/rasff-window/portal/?event=searchForm&cleanSearch=1
Search keywords = benzo(a)pyrene, Ghana, smoked fish

2.3 CONCLUSION

The findings suggest that PAHs are the most important food safety hazards associated with the processing technique and handling of traditionally smoked fish in Ghana. The findings also suggest that post-processing contamination is an important contributor to the hazard levels in the products. Investments should therefore be made in improving the existing fish smoking techniques and/or ensuring the use of improved techniques that have been demonstrated to be efficacious. Support for, education on, and enforcement of regulations on good post-processing handling of smoked fish are recommended. The hazard scoring approach presented in the study yielded the same output as the decision flowchart in terms of identifying the most important hazard, and also provided additional insights about other hazards (such as occurrence suggesting hygiene lapses) that are missed when the flowchart is used. The proposed method may thus find application for screening hazards for relevance in situations where hazard concentration and prevalence data are available.

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Having determined in this Chapter that PAHs are the most important food safety hazards in traditionally smoked fish and considering that the FTT was introduced primarily to address that hazard, the second objective of this study was to evaluate the efficacy of the FTT through comparative fish smoking experiments with the Chorkor smoker and the metal drum oven. This is presented in Chapter 3.

CHAPTER 3: EFFICACY OF THE FTT TO REDUCE PAH CONTAMINATION OF SMOKED FISH

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ABSTRACT

This observational study investigated the efficacy of the FTT as an intervention for reducing PAH contamination of smoked fish. *Sardinella* sp. and *Sphyraena* sp. were smoked separately on the FTT, the Chorkor smoker and the metal drum oven and their BaP and PAH4 contents were determined by gas chromatography-mass spectrometry. The effect of processing fuel type on PAH contamination was also determined. The mean BaP and PAH4 levels in smoked fish from the FTT were up to 2 ± 1 µg/kg and 8 ± 3 µg/kg, respectively. The Chorkor smoker products had up to 61 ± 6 µg/kg for BaP and 395 ± 17 µg/kg for PAH4. For the metal drum, the values were 70 ± 4 µg/kg for BaP and 327 ± 37 µg/kg for PAH4. Thus, whereas PAH levels in FTT products were below EU regulatory limits (2 µg/kg for BaP and 12 µg/kg for PAH4), levels in products of the traditional ovens exceeded these limits by up to 33 times. Use of fuelwood caused higher PAH contamination than the use of charcoal as processing fuel. The findings suggest that the FTT is efficacious in reducing PAH contamination of fish during smoking, and that fuel type and oven design impact the extent of the contamination

Keywords: Smoked fish, polycyclic aromatic hydrocarbons, smoking ovens, food safety

3.0 INTRODUCTION

As discussed in Chapters 1 and 2, the main reason for the development of the FTT was to address the problem of high PAH contamination of smoked fish in the global South (Ndiaye and Diei-Ouadi, 2011; FAO, 2014; Ndiaye et al., 2015; Peñarubia et al., 2017; Mindjimba et al., 2019). The design characteristics of the FTT (Chapter 1) are in line with the Codex guidelines for reducing PAH in smoked products (CAC, 2009, 2012). The cited Codex guidelines identify the use of fuelwood, distance between the food being smoked and the heat source, and smoking duration as important parameters affecting the levels of the hazard in smoked products. It was expected, therefore, that smoked products from the innovation will record significantly lower PAH levels than those from traditional and first-generation improved ovens. This observational study investigated the efficacy of the FTT in that regard. Specifically, the impact of oven design, fuel type, and smoked product type on PAH levels were tested through comparative fish smoking experiments among the FTT, the Chorkor smoker and the metal drum oven.

3.1 MATERIALS AND METHODS

3.1.1 Fish species and product types

The selected fish species were *Sardinella* sp. and *Sphyraena* sp., two well-known species in Ghana. These were processed into smoked-soft and smoked-dry products (described in Chapter 1, Section 1.3).

3.1.2 Smoking experiments

Three sets of smoking experiments were conducted according to the general process flow in Fig. 1.8 (Chapter 1). Smoking sessions were conducted by women career processors with over three decades of experience, on actual ovens (not laboratory-scale equipment) under the observation of the researcher. Each experiment was conducted in five replicates. For each replicate, 18 kg of raw fish was smoked.

3.1.2.1 Fuel types

Conventionally, dry fuelwood from several tree species are used for traditional fish smoking in Ghana (Nerquaye-Tetteh et al., 2002). Two of the most patronized species are *Pterocarpus erinaceous* (Senegal rosewood, locally called "*esa*") and *Azadirachta indica* (neem) (Nerquaye-Tetteh et al., 2002; Ahadzi, 2017). *P. erinaceous* is listed as an endangered tree species by the International Union for Conservation of Nature (Barstow, 2018). As explained in Chapter 1, for the FTT, a mixture of charcoal and heat-retention stones are used as fuel for the cooking step, and sugarcane bagasse as fuel for the smoke-flavouring step (Ndiaye et al., 2015). The named fuels were used in different combinations on the different ovens, depending on the test objective.

3.1.2.2 Smoking experiments I: comparison of PAH levels among products of the three ovens

This set evaluated the performance of the FTT, the Chorkor smoker and the metal drum oven as different *systems* for fish smoking vis-à-vis PAH levels in the products. Each oven was operated as originally intended to be used. Thus, the FTT was fuelled with charcoal and broken pottery for the cooking step, and with sugarcane bagasse for the smoke flavouring, while the Chorkor smoker and the metal drum oven were fuelled with *P. erinaceous* fuelwood.

3.1.2.3 Smoking experiments II: impact of fuel type on PAH level

In this set of experiments, the effect of processing fuel type on PAH levels in smoked-dry *Sardinella* sp. was evaluated across the three ovens. The two wood species (*P. erinaceous* and *A. indica*) were used to fuel each oven in separate experiments. The Chorkor smoker was also operated to mimic the FTT by using fully-lit charcoal and broken pottery to first cook fish, followed by flavouring with sugarcane bagasse smoke.

3.1.2.4 Smoking experiments III: impact of FTT parts on PAH levels

The final set of smoking experiments was conducted on the FTT only. It determined the effects of the various parts of the FTT on PAH levels in smoked-dry *Sardinella* sp.

3.1.2.4.1 Impact of the FTT fat collector on PAH levels

The fat collector of the FTT prevents fish fat from dripping into the heat source during processing. The impact of this appendage on PAH level was tested by sampling at the end of the cooking step with the tray in place (five replicates) and without the tray in place (5 replicates).

3.1.2.4.2 Impact smoke flavouring material on PAH levels

Batches of *Sardinella* sp. cooked into a dry product on the FTT were flavoured separately with sugarcane bagasse, coconut husk or *A. indica* smoke, and their PAH levels determined. Sugarcane bagasse and coconut husk were listed by the innovators of the FTT as suitable fuels for flavouring products (Ndiaye et al., 2015), hence their use in the tests. *A*. *indica* was also testíg0 to evaluate the impact of using fuelwood as the smoke-flavour source. Experiments were conducted in five replicates for each material.

3.1.2.4.3 Impact of the FTT smoke filter on PAH levels

This was tested by flavouring smoked-dry *Sardinella* sp*.* with and without the filter in place, after the cooking step. Smoke for flavouring was generated from sugarcane bagasse and *A. indica*. Filter use for each smoke material was tested in five replicates.

3.1.3 Laboratory analysis of PAH

Smoked samples were homogenized, vacuum-packed and frozen (-22ºC) in Ghana until shipped by air to Belgium for analysis. The same sampling and analytical procedures described in Chapter 2 were applied (Sections 2.1.3.1 and 2.1.5).

3.2 Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences software (SPSS®, version 22) and Microsoft Excel® 2016. In computing mean PAH levels, the LOQ value (0.20 µg/kg) was substituted for concentrations below the LOQ. Raw data were tested for normality by the Shapiro-Wilk test. Means of normally distributed data were compared by independent t-tests and ANOVA, as appropriate. Non-parametric tests were used to compare means of data that were not normally distributed.

3.3 RESULTS AND DISCUSSION

In the following sections, only BaP and PAH4 values are discussed, since those two are used as markers for regulating PAHs (Chapter 1; EFSA, 2008). However, data on benzo(a)anthracene, chrysene and benzo(b)fluoranthene are provided in Appendix 1. Results of the three experimental smoking sets are reported in Tables 3.1 – 3.4.

3.3.1 FTT vs. traditional ovens as smoking systems (Experiment Set I)

Among the three ovens, FTT products had the lowest mean PAH levels (Table 3.1). BaP and PAH4 in FTT products were up to 215 and 183 times lower, respectively, than in Chorkor smoker products, and 91 and 63 times lower, respectively, than in metal drum products. FTT products also had lower PAH levels than the EU limits for BaP (2 µg/kg) and PAH4 (12 µg/kg) (Regulation (EC) No. 835/2011). For traditional oven products, mean BaP and PAH4 levels exceeded the EU limits by up to 35 times and 33 times, respectively (Table 3.1). These, therefore, suggest that, as a (fish) smoking system, FTT yields products with significantly lower PAH levels than the Chorkor smoker and the metal drum oven. Fig. 3.1 shows the appearance of smoked-dry *Sardinella* sp. produced on each of the ovens. The intensity of their colour indicates the extent of smoke deposition on the products, which also corresponded with the PAH contamination levels. The Chorkor smoker product (darkest) had the highest PAH levels, whereas the FTT product (dark) had the lowest PAH levels (Table 3.1). The metal drum product (darker) was between the two in PAH contamination level (Table 3.1).

The occurrence of elevated PAH levels in the Chorkor smoker and the metal drum oven products and the differences between them were discussed in Chapter 2, Section 2.3. It was shown that the Chorkor smoker products had higher ($p<0.05$) PAH levels than the metal drum oven products.

Fig. 3.1: Appearance of smoked-dry *Sardinella* sp. produced on the FTT (a), the metal drum oven (b) and the Chorkor smoker (c)

3.3.2 Effect of smoked product type on PAH level (from data of Experiment Set I)

As observed in Chapter 2, smoked-dry products had higher ($p<0.05$) PAH levels than their corresponding smoked-soft forms (Table 3.2), due to the longer exposure of the former to heat and smoke during processing (Stołyhwo and Sikorski, 2005).

3.3.3 Effect of fuel type and oven design on PAH levels (Experiment Set II)

The fuelwoods caused significantly higher ($p<0.05$) PAH contamination than charcoal (Table 3.3). Between the two fuelwoods, *A*. *indica* caused higher ($p<0.05$) PAH contamination than *P. erinaceous*, except in the case of BaP for the Chorkor smoker, where no differences ($p>0.05$) were found. On the FTT, BaP in fish processed with *P. erinaceous* and *A. indica* were 1.9 ± 0.2µg/kg and 7.7 ± 0.4µg/kg (mean ± stdev), respectively, representing 10- and 39-fold higher hazard levels than in fish processed with charcoal (Table 3.3). Thus, although the design of the FTT allows for reduction of PAH contamination, use of fuelwood compromises the efficacy of the oven. Smoke release from the fuelwood (which is not observed with fully-lit charcoal) could be cited to account for the difference (Simko, 2002).

On the Chorkor smoker, use of charcoal in place of the fuelwoods resulted in a six-fold reduction in BaP contamination and up to a 10-fold reduction in PAH4 (Table 3.3). Thus, although the design of the traditional and first-generation improved ovens enhances PAH contamination (CAC, 2009), use of non-woody fuels reduced the extent of the contamination. This, together with the observation made on the FTT, shows that *both* oven design and fuel type affect PAH contamination levels.

Table 3.1: Mean BaP and PAH4 levels in *Sardinella* sp. and *Sphyraena* sp. smoked on the FTT and traditional ovens (Experiment Set I. Each experiment was conducted in 5 replicates. Data for Chorkor smoker and Metal drum oven from Chapter 2)

Product	Oven	Fuel[†]	Mean ± stdev BaP (μg/kg)	Mean ± stdev PAH4 (μg/kg)
Raw *Sardinella* sp.	-	-	ND	ND
Raw *Sphyraena* sp.	-	-	ND	ND
Smoked-soft *Sardinella* sp.	FTT	Charcoal (for cooking) and sugarcane bagasse (for smoke- flavouring)	0.2 ± 0.0 [a‡]	1.5 ± 0.2[a]
	Chorkor smoker	*Pterocarpus erinaceous*	26 ± 5[b]	167 ± 6[b]
	Metal drum oven	*Pterocarpus erinaceous*	11 ± 2.[c]	58 ± 4[c]
Smoked-dry *Sardinella* sp.	FTT	Charcoal (for cooking) and sugarcane bagasse (for smoke-flavouring)	0.3 ± 0.2[a]	2.2 ± 0.7[a]
	Chorkor smoker	*Pterocarpus erinaceous*	60 ± 4[b]	395 ± 17[b]
	Metal drum oven	*Pterocarpus erinaceous*	26 ± 2[c]	136 ± 11[c]
Smoked-soft *Sphyraena* sp.	FTT	Charcoal (for cooking) and sugarcane bagasse (for smoke- flavouring)	0.6 ± 0.2[a]	3.6 ± 0.9[a]
	Chorkor smoker	*Pterocarpus erinaceous*	50 ± 1[b]	270 ± 10[b]
	Metal drum oven	*Pterocarpus erinaceous*	37 ± 7[c]	168 ± 33[c]
Smoked-dry *Sphyraena* sp.	FTT	Charcoal (for cooking) and sugarcane bagasse (for smoke- flavouring)	2 ± 1[a]	8 ± 3[a]
	Chorkor smoker	*Pterocarpus erinaceous*	61 ± 6[b]	360 ± 29[b]
	Metal drum oven	*Pterocarpus erinaceous*	70 ± 4[b]	327 ± 34[c]

For each product, means in columns with different superscripts are significantly different ($p<0.05$) among the ovens
[†]On the FTT oven, fully-lit charcoal combined with broken clay pottery was used as heat source for the cooking step
ND= not detected
[‡]Values for 3 of 5 samples were below LOQ (0.2 μg/kg). Values for remaining 2 samples were equal to the LOQ

Table 3.2: Mean PAHs in smoked-soft products vs. smoked-dry products (products from all oven types considered, from data of Experiment Set I)

Oven	Mean ± stdev BaP (µg/kg)		Mean ± stdev PAH4 (µg/kg)	
	Smoked-soft products	Smoked-dry products	Smoked-soft products	Smoked-dry products
FTT	0.4 ± 0.20[a]	1.0 ± 0.9[b]	3 ± 1[a]	4.6 ± 4[b]
Chorkor smoker	38 ±13[a]	61 ± 5[b]	219 ± 52[a]	377 ± 29[b]
Metal drum oven	24 ±14[a]	48 ± 22[b]	113 ± 60[a]	231 ± 99[b]

For each oven, BaP and PAH4 with different superscripts are significantly different ($p<0.05$)

Table 3.3: Effect of fuel type on PAH levels in smoked-dry *Sardinella* sp. produced on the FTT and traditional ovens (Experiment Set II)

Oven	Fuel	Mean ± stdev BaP (µg/kg)	Mean ± stdev PAH4 (µg/kg)
FTT	Charcoal† (for cooking) and sugarcane bagasse (for smoke-flavouring)	0.2 ± 0[a ‡]	1.5 ± 0.2[a]
	Azadirachta indica for both cooking and smoke-flavouring	7.7 ± 0.4[b]	29 ± 2.[b]
	Pterocarpus erinaceous for both cooking and smoke-flavouring	1.9 ± 0.2[c]	37 ± 3[c]
Chorkor smoker	Charcoal (for cooking) and sugarcane bagasse (for smoke-flavouring)	10.2 ± 0.4[a]	39 ± 2[a]
	Azadirachta indica	59 ± 7[b]	207 ± 10[b]
	Pterocarpus erinaceous	60 ± 4[b]	395 ± 17[c]
Metal drum oven	*Azadirachta indica*`	36 ± 4[a]	174 ± 23[a]
	Pterocarpus erinaceous	26 ± 2[b]	136 ± 11[b]

For each oven, BaP and PAH4 with different superscripts are significantly different ($p<0.05$) among the ovens

†Fully-lit charcoal combined with broken pottery was used as heat source for the cooking step

‡Values for 3 of 5 samples were below LOQ (0.2µg/kg). Values for remaining 2 samples were equal to the LOQ

3.3.4 Effect of FTT Parts on PAH levels (Experiment Set III)

Having determined that smoked products from the FTT had lower PAHs levels than those from the traditional ovens regardless of fuel type, the effects of the components of the FTT on levels of the hazard were evaluated. For these experiments, smoked-dry *Sardinella* sp. was considered, since smoked-dry products had higher PAH levels than the smoked-soft forms due to the longer heat and smoke exposure (Table 3.2).

3.3.4.1 Use or non-use of the fat collector

When the fat collector was not used, BaP and PAH levels were respectively six- and eight-times higher ($p<0.05$) than the values when it was used (Table 3.4). EU limits were also violated (2.9 ± 0.2 µg/kg for product vs 2.0µg/kg limit for BaP; 39 ± 2 µg/kg product vs. 12 µg/kg limit for PAH4). This could be accounted for by two factors. First, when the collector is not used, fish fat drips unto the heat source and increases PAH production through pyrolysis of the fat (Fig. 3.2) (Rengarajan et al., 2008). Secondly, without the collector, there is a direct contact between the heat source and the products. Both factors are considered important contributors to PAH contamination during smoking processes (CAC, 2009).

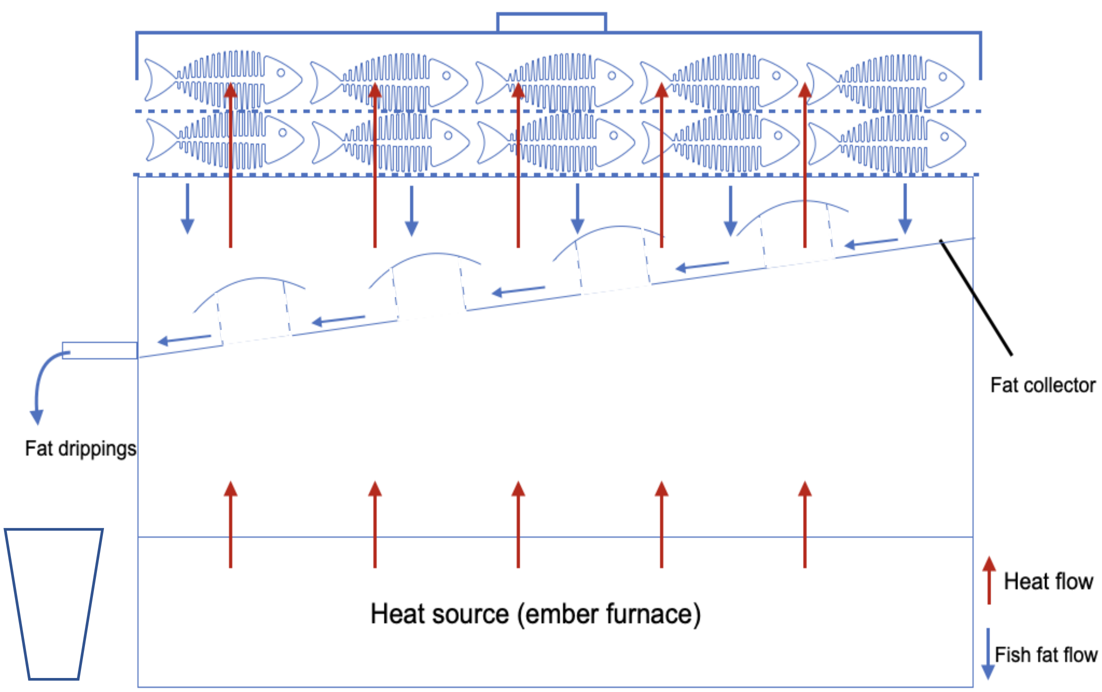

Fig. 3.2: An illustrated transverse section of an FTT oven to show indirect heat flow to fish and fat drippings flow from fish during smoking on the FTT (also shown in Chapter 1)

The fat collector, therefore, contributes to the observed lower PAHs in FTT products by reducing fat pyrolysis and allowing an indirect heat transfer to fish during processing (Fig. 3.2).

3.3.4.2 Type of material for generating smoke to flavour fish

The three materials tested (sugarcane bagasse, coconut husk and *A. indica* (Fig. 3.3) yielded significantly different ($p<0.05$) BaP and PAH4 levels (Table 3.4). Products flavoured with *A. indica* and sugarcane bagasse smoke recorded the highest and lowest PAH levels, respectively (Table 3.4). Ndiaye et al., (2015) classified certain fuels for the FTT by three colours: green (highly recommended), orange (use with caution) and red (avoid). In that classification, sugarcane bagasse was marked green, coconut husk orange and woody fuels red. The findings of the present study thus accord with that classification. Another study in Ghana found that sugarcane bagasse smoke yielded lower PAHs than hard wood when used to flavour fish (Essumang et al., 2013).

The differences notwithstanding, the EU regulatory limits were not exceeded for any of the materials. Hence, the practical relevance of the statistical differences is minimal since none of the materials raised PAH contamination above acceptable levels.

(a) (b) (c) (d)

Fig. 3.3: Materials for generating smoke (a=Sugarcane bagasse; b= coconut husk; c= *A. indica* sticks) and smoke-generation for indirect smoke-flavouring on the FTT (d)

3.3.4.3 Use or non-use of the filter

When the smoke filter was not used, BaP and PAH4 levels were higher by up to four- and five times, respectively (Table 3.4). The EU regulatory limits were, however, not violated, except in the case of *A. indica* smoke where PAH4 levels were marginally higher than the limit (13 µg/kg in product vs. 12 µg/kg limit) (Table 3.4). Fig. 3.4 shows that the filter trapped tar particles that may otherwise have been deposited on the products to increase PAH content. Essumang et al., (2013) observed that filtering wood smoke reduced PAH levels in products from a traditional oven in Ghana to which an activated charcoal filter was affixed. Although the oven described in that study differs operationally from the FTT, their finding supports the lessening effect of smoke filtering on PAH contamination. The use of luffa sponge (a readily available, renewable resource) as done on the FTT may, however, be considered more economically and environmentally sustainable than the activated charcoal option described by Essumang et al. (2013).

(a) Filter unit of the FTT

(b) Filter Before use

(c) Filter After use: Front (facing smoke stream)

d) Filter after use: Back

Fig. 3.4: FTT smoke filter before and after use for one smoking session

Table 3.4: Effect of FTT parts on PAH levels in smoked-dry *Sardinella* sp. (Experiment Set III. Each experiment was conducted in 5 replicates)

Test	Condition/Material		Mean ± stdev BaP (µg/kg)	Mean ± stdev PAH4 (µg/kg)
Effect of fat collector†	Fat collector used		0.5 ± 0.2[a]	4.7 ± 0.1[a]
	Fat collector not used		2.9 ± 0.2[b]	39 ± 2[b]
Effect of type of material for generating smoke to flavour products	Sugarcane bagasse		0.2 ± 0[a]	1.5 ± 0.2[a]
	Coconut husk		0.3 ± 0.2[b]	2 ± 1[b]
	A. indica		0.8 ± 0.3[c]	8 ± 1.[c]
Effect of smoke filter	Sugarcane bagasse smoke	Filter used	0.2 ± 0[a]	1.5 ± 0.2[a]
		Filter not used	0.9 ± 0.2[b]	8.0 ± 0.3[b]
	A. indica smoke	Filter used	0.8 ± 0.3[a]	8 ± 2[a]
		Filter not used	1.4 ± 0.2[b]	13 ± 1[b]

For all experiments, charcoal combined with broken pottery was used as cooking fuel.
For each test, means in columns with different superscripts are significantly different ($p<0.05$)
†Smoke-flavouring was not applied

3.3.4 CONCLUSION

The findings suggest that the FTT is efficacious in yielding smoked products with PAH levels that are lower than those in the Chorkor smoker and metal drum oven products. Good practices for ensuring low PAH contamination when operating the FTT are using fully-lit charcoal with broken pottery as heat source, keeping the fat collector in place. Under those conditions, PAH levels in FTT products are lower than current EU limits (Regulation (EC) No. 835/2011). Since non-use of the filter did not raise PAH contamination beyond the regulatory limit, the smoke-generation and filtering unit of the FTT may be considered redundant. Eliminating this appendage could be helpful in reducing the construction cost of the FTT.

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In the next chapter, the extent to which the reduced PAH contamination in FTT products translates into public health protection in the Ghanaian context is evaluated from a risk assessment perspective.

CHAPTER 4: RISK ASSESSMENT OF PAHs IN SMOKED *SARDINELLA* SP. IN GHANA: IMPACT OF THE FTT ON PUBLIC HEALTH PROTECTION

Redrafted from:

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ABSTRACT

This study examined consumer exposure to PAHs in smoked *Sardinella* sp. in Ghana and evaluated the extent to which the use of the FTT could reduce such exposure (*Sardinella* sp. is the most consumed fish species in Ghana). The product was sampled from selected markets in the southern, central and northern regions of the country and also directly from the FTT, the Chorkor smoker and the metal drum oven at a processing site, and tested for PAH levels. By cross-sectional consumer surveys in the same regions, the intakes of the product (frequencies of consumption and quantities thereof) were determined. Using the hazard concentration and consumption data, a probabilistic risk assessment of PAH was carried out by the margin of exposure (MoE) approach. MoE values as low as 260 and 572 were obtained for products from the traditional ovens and the markets, respectively, whereas the lowest value for FTT products was approximately 72,000. For PAHs, an MoE less than 10,000 denotes a serious public health concern. Therefore, the findings suggest that there is a potential health concern of high consumer exposure to PAHs in traditionally smoked fish in Ghana, and that the FTT is an effective technical intervention for the problem.

Keywords: polycyclic aromatic hydrocarbons; fish smoking ovens; food safety; risk assessment

4.0 INTRODUCTION

In Chapter 3, it was demonstrated via comparative smoking experiments that the BaP and PAH4 levels in smoked fish from the FTT were over 200 times and 180 times, respectively, less than the corresponding levels in products of the traditional ovens (Chorkor smoker and the metal drum oven). The efficacy of the FTT alone, however, may not provide sufficient grounds for the adoption of the innovation. It is important to first establish the potential public health impact of the consumption of smoked products from the traditional ovens, followed by determination of the potential impact of the FTT on the situation.

In general, high food safety hazard levels may not necessarily translate into concerns for public health (Roesel and Grace, 2015). Studies have shown that although there is a high prevalence of food safety hazards in products on sub-Saharan Africa markets, risks for illness for many of the hazards are low due to factors such as food consumption habits and food preparation practices (Grace et al., 2015). It is important, then, to determine the public health burden associated with the elevated PAH levels in products of the Chorkor smoker and the metal drum oven, as well as the impact of the FTT oven on the situation. Such an evaluation requires risk assessment.

Risk assessment in food safety is a scientific process that uses available, credible evidence to estimate the likelihood and severity of an adverse health effect occurring when a food safety hazard is ingested (CAC, 1999; Williams et al., 2000; FAO/WHO, 20009). It involves evaluation of the potential of a substance in food to cause adverse health effects and the mechanism by which it does so (hazard identification), determination (qualitative or quantitative) of the severity of the adverse health effects in a dose-response relationship (hazard characterisation), determination of the likely intake of the hazard through food (exposure assessment)(CAC, 1999), and a final determination of the nature and magnitude of the risk based on the outputs of the preceding three steps (risk characterization) (CAC, 1999; FAO/WHO, 2009; Barlow and Schlatter, 2010; Hanlon et al., 2016).

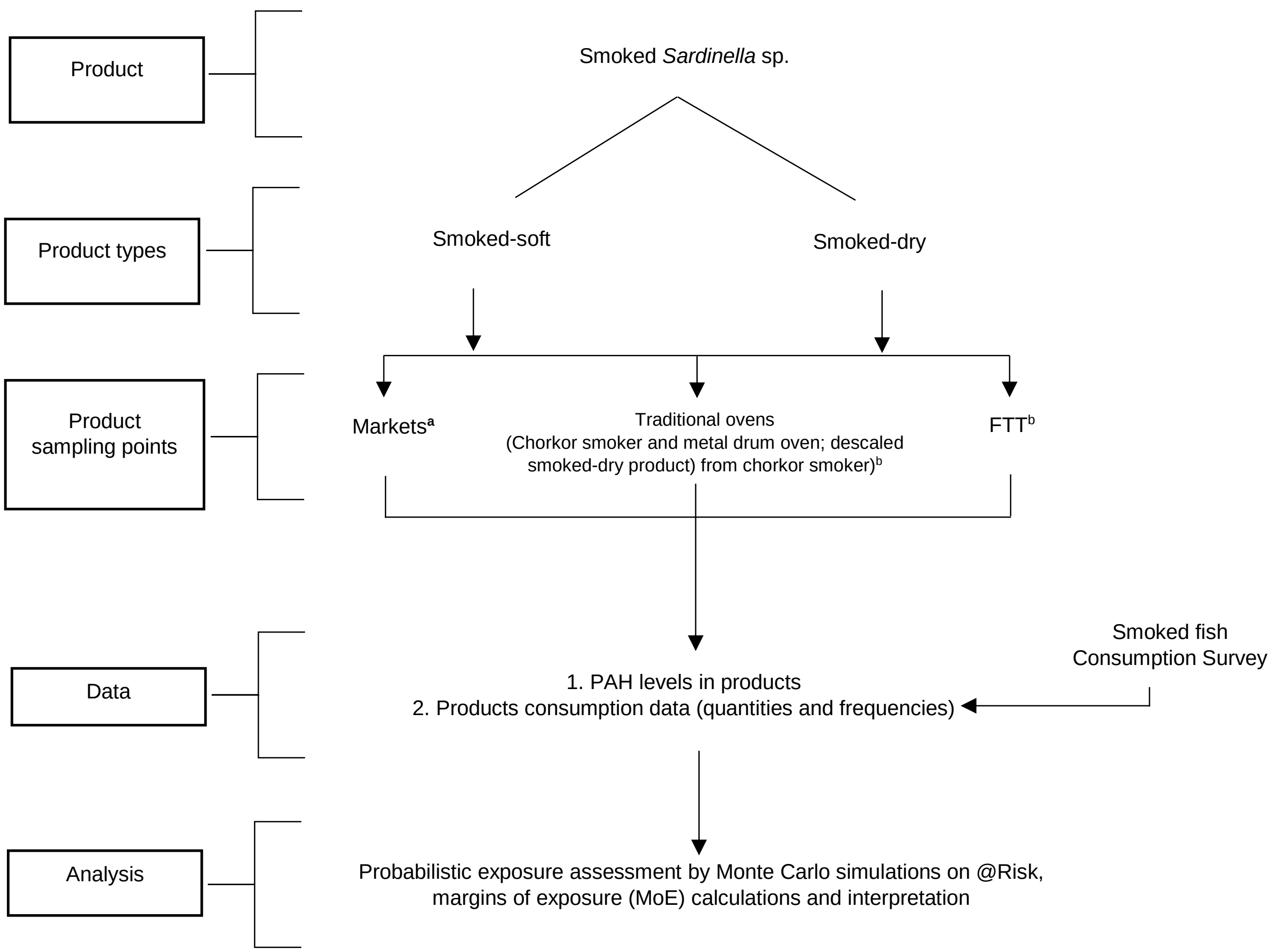
As noted in Chapter 1, PAHs are identified as food safety hazards with both mutagenic and carcinogenic potential (Ciecierska and Obiedziński 2007; Ravindra et al., 2008; Benford et al. 2010), with the the International Agency for Research on Cancer (IARC) classifying BaP as a human carcinogen (Group 1) and benzo(a)anthracene, chrysene, and benzo(b)fluoranthene as probable human carcinogens (Group 2B) (IARC 2018). Concerning hazard characterization, the compounds are known to have a non-threshold effect; that is, any dose could potentially cause the adverse health effect (Williams et al., 2000; Barlow and Schlatter, 2010; see Chapter 1). As a result, approaches for hazard characterization of PAHs consider the extent to which the intake levels (exposure) approximate doses that produced low but measurable deleterious effects in experimental rats (Barlow et al., 2006; Cartus and Shrenk, 2017). This approach, called the margin of exposure (explained further in Section 4.1.6), provides an indication of the level of risk management concern needed for the hazard in particular contexts (EFSA, 2005).

The MoE approach has been applied by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and by EFSA's Scientific Panel on Contaminants in the Food Chain (EFSA, 2008) to conduct risk assessment not only for PAHs, but also for acrylamide, ethyl carbamate, 1,3-dichloro-2-propanol, and aflatoxins (Barlow and Schlatter, 2010). It estimates the intakes of PAHs in a given food, in a given population, and compares the output with the level that caused the exposure end-point (cancer) in experimental animals. Since the latter is usually established (i.e. information available in literature), the remaining task is the determination of consumer exposure to PAHs in the food of interest and in the context of interest.

In this study, the risk associated with the high PAH levels in traditionally smoked fish was determined, and the effect of a consumer-level food preparation practice on such risk also evaluated. The effect of the low PAH levels in FTT products on consumer exposure to the hazards was then determined.

4.1 MATERIALS AND METHODS

A schematic overview of the study methodology is shown in Fig. 4.1. Smoked-soft and smoked-dry *Sardinella* sp. were sampled from three sources: markets, directly from traditional ovens (the Chorkor smoker and the metal drum oven), and directly from the FTT (Fig. 4.1) and analysed for their PAH levels. A consumption survey was conducted to determine the quantities and frequencies of intake of the products in the southern, central and northern parts of Ghana. A probabilistic chronic exposure assessment for the hazard was then conducted using the PAH contamination data and the smoked fish consumption data, after which margins of exposure were calculated and discussed.



a: Smoked fish on markets in Ghana are processed on traditional ovens. However, the characteristic lack of traceability on the markets makes it difficult to establish which specific traditional oven (i.e. whether Chorkor smoker or metal-drum) was used to process a given consignment of smoked fish. Sampling from these markets allowed determination of the prevailing PAH exposure, since consumers obtain the products from the markets.
b: Samples were collected directly at a processing site, immediately after smoking

Fig. 4.1: Schematic overview of the study methodology

4.1.1 Study areas

Three of the ten regions[2] in Ghana were so selected to allow a geographical coverage of the country from the south to the north and thus provide a national overview of the situation under study. The selected regions were Greater Accra (a southern, coastal region important for fishing, traditional fish processing and smoked fish trade and home to the capital city, Accra. It was also the only region with an installed FTT at the time of the study); Brong Ahafo (a centrally located, landlocked region and important hub for smoked fish trade); and Upper East (a northern, landlocked region). The specific towns/suburbs in each region for the study were Madina, Adabraka and Tema New Town in the Greater Accra Region; Sunyani and Techiman in the Brong Ahafo Region; and Bolgatanga and Navrongo in the Upper East Region.

4.1.2 Fish species and product types

Smoked-soft and smoked-dry *Sardinella* sp. were used for the study. (Differences between the products are explained in Chapter 1).

Some consumers remove the skin of some smoked fish products during food preparation[3]. The impact of such treatment on PAH contamination was determined by descaling smoked-dried *Sardinella* sp. produced on the Chorkor smoker and analysing it for PAH content.

4.1.3 Survey on consumption of smoked *Sardinella* sp.

With semi-structured questionnaires, cross-sectional surveys were conducted by direct interviews with anonymized individuals in households in the study areas. A total of 900 respondents were interviewed. Sample sizes were determined using the OpenEpi online toolkit (Dean et al., 2013). Inputs for the toolkit were the population data of the study areas (from the Ghana 2010 census) (GSS, 2012) and a confidence level of 95%. Outputs were rounded up to the nearest hundred. The resulting sample sizes were 300 for each region.

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[2] At the time of the data collection, Ghana was divided into ten administrative regions. The country has since been re-organized into 16 regions.

[3] This is not a common practice in Ghana, and very few fish species can be treated this way when smoked.

Inclusion criteria were that respondents be willing to participate, be consumers of smoked *Sardinella* sp., be permanent residents in the survey areas and be at least 16 years old (to keep the focus on the adult population). Consumers estimated their portion sizes as multiples of a 50g smoked-dry *Sardinella* sp. exhibit shown to them during the interviews.

4.1.4 Smoked fish sampling points

The products were sampled from the following points:
a) markets: Smoked fish on markets in Ghana are invariably processed on either the Chorkor smoker or the metal drum oven. However, due to the characteristic lack of traceability on the markets (Roesel and Grace, 2015), it is practically difficult to establish the specific type of traditional oven (i.e. whether Chorkor smoker or metal drum oven) that was used to process a given consignment of smoked fish. Sampling at the markets was to enable determination of the prevailing PAH exposure in the products under study.

The selected markets were the Adabraka Fish Market and the Madina market in the Greater Accra Region (PAH data already discussed in Chapter 2); Sunyani and Techiman markets in the Brong Ahafo Region; and Bolgatanga and Navrongo markets in the Upper East Region. A total of 60 samples were purchased across the regions (1 fish species x 2 product types x 6 markets x 5 sampling rounds = 60 samples).

b) traditional ovens: products were collected directly from the Chorkor smoker and the metal drum oven immediately after smoking, at a processing site in Tema New Town in the Greater Accra Region (Data from Chapter 2).

c) the FTT: products were sampled directly from the FTT immediately after processing. (Data from Chapter 3).

For sampling points *b)* and *c)*, details of the fish smoking processes were described in Chapters 2 and 3.

4.1.5 Analysis of PAH

Smoked samples were homogenized, vacuum packed and kept frozen (-22°C) in Ghana until shipped by air to Belgium for analysis. The same sampling and analytical procedures described in Chapter 2 were applied.

4.1.6 Data analysis and exposure calculations

The raw data of the consumer survey and PAH concentrations were tested for normality by the Shapiro-Wilk test. Means of normally distributed data were compared by independent t-tests and ANOVA, as appropriate. Nonparametric tests were used to compare means of data that were not normally distributed. These tests were ran using SPSS, version 22 (IBM Corp., Armonk, NY, USA).

4.1.6.1 Calculation of usual intake of Sardinella sp.

For each product (smoked-soft or smoked dry *Sardinella* sp.), the quantity consumed per day and per person (in g/day.person) was calculated as:

$$Q = \left(\frac{n}{N}\right) x\, P \quad (1)$$

where:

Q = reported quantity consumed per day per person (g/day.person)

n= reported number of times product is consumed per period (“period” = daily, weekly or monthly)

N = reported number of days in period

P = reported portion size based on 50g exhibit (g)

For example, for a consumer who reported usual intake of 100g (i.e. 2 portions of the 50g exhibit) of smoked-soft *Sardinella* sp. once a week, Q was calculated as

$$\left(\frac{1}{7}\right) x\ 100g = 14.3\text{g/day.person.}$$

In this way, Q for each product was calculated for each consumer in each region as the usual intake of the product. Each Q(g/day.person) was converted to Q(kg/day.person) by dividing by 1000. Subsequently, the Q(kg/day.person) values were expressed per body weight as

$$Q\ (kg/kg\ bw/day) = Q\ (kg/day.person) \div Body\ weight\ (kg) \quad (2)$$

An adult body weight of 60kg was assumed for all respondents (Walpole et al., 2012).

4.1.6.2 Best-fit distributions for consumption data and PAH levels

For a probabilistic estimation of PAH exposure, best-fit distributions based on Chi square statistic considering percentile-percentile and quantile-quantile plots were determined for Q (kg/kg bw/day) of each product in each region using @Risk version 7 (Palisade Corporation, Ithaca, NY, USA). The same was done for the PAH concentrations (C) for each product.

4.1.6.3 Probabilistic estimation of PAH exposure

PAH exposure for each product was estimated from the distribution functions of Q and C as:

$$E = Q_d \times C \quad (3)$$

where:

E = PAH exposure (µg/kg bw/day)

Q_d = distribution function of Q (kg/kg bw/day)

C = distribution function of PAH level (µg/kg)

Monte Carlo simulations were ran on the output of equation (3) at 50,000 iterations with @Risk version 7 (Palisade Corporation, Ithaca, NY, USA). Three simulations were ran to confirm the stability of outputs. Since the usual dietary intakes of the products were considered, the E values represent chronic exposure to PAHs in the products.

Three exposure scenarios were evaluated as follows:

i. *Scenario 1 (market products)*: This involved estimation of PAH exposure using C for products from the markets in each region and Q_d for the same products in the corresponding regions. This scenario represents the status of PAH exposure in traditionally smoked *Sardinella* sp. from Ghanaian markets at the time of the study.

ii. *Scenario 2 (traditional ovens vs. FTT):* Exposure was estimated using the consumption data (Q_d) for Greater Accra Region and C in the corresponding products from the three ovens. This served to measure differences in PAH exposure between traditional oven products and FTT products and also show the impact of the latter as an intervention.

iii. *Scenario 3 (impact of a food preparation step):* The exposure in this scenario was determined using Q_d for Greater Accra region and C for the descaled smoked-dry *Sardinella* sp.

In each scenario, exposure was estimated separately for BaP and PAH4.

4.1.6.4 Determination of margin of exposure (MoE)

For chemical hazards with non-threshold genotoxic effects (such as PAHs), both the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) recommend the margin of exposure (MoE) approach for risk characterization (JECFA, 2005; EFSA, 2005; O'Brien et al., 2006; Barlow and Schlatter, 2010). This approach estimates public health risk by comparing the dietary intakes of a given hazard in a given human population with a level that produced an adverse health effect in animal studies (Benford et al., 2010). It is defined as the ratio between the lower limit of the benchmark dose level ($BMDL_{10}$) from animal studies to the estimated exposure in a given human population (EFSA, 2005). The resulting MoE value is a dimensionless number that does not give a precise quantitative risk estimate but provides an indication of the level of risk management attention needed for the hazard considered (Cartus and Schrenk, 2017).

MoE was estimated as:

$$\mathrm{MoE} = \frac{\mathrm{BMDL_{10}}}{\mathrm{E}} \qquad (4)$$

$BMDL_{10}$ of 100µg/kg bw/day and 340µg/kg bw/day have been determined for BaP and PAH4, respectively (EFSA, 2005; JECFA, 2005). Those $BMDL_{10}$ values and the outputs of exposure (E, from equation 3) for each PAH-product pair were substituted into equation 4 to determine the corresponding MoE.

For carcinogenic compounds, MoE lower than 10,000 is considered to denote a high public health concern that should be prioritized for risk management (EFSA, 2005), whiles an MoE higher than 10,000 would be of low concern from a public health point of view and thus make the hazard a low priority for risk management actions (EFSA, 2005). The value of 10,000 was based on considerations of species differences and human variability in toxicokinetics and toxicodynamics, human variability in cell cycle control and capacity for DNA repair (EFSA 2005; Barlow and Schlatter, 2010). In the interest of public health, even at MoE above 10,000, it is recommended that risk managers do not preclude the application of the necessary measures to reduce human exposure hazards (Barlow et al., 2006).

4.2 RESULTS AND DISCUSSION

4.2.1 Consumption of smoked *Sardinella* sp.

Out of the survey sample size of 900, a total of 812 valid responses were obtained (90.2% response rate), comprising 212 from Greater Accra Region, 300 from the Brong Ahafo Region and 300 from the Upper East Region. The mean intakes (g/day.person) of the products are presented in Fig 4.1. The data represented by the figure does not consider age and gender differences, since the study objective focuses on overall consumption for the general adult population. However, demographic differences are provided in Appendix 2. The details therein do not alter the risk assessment outcome of comparing exposure between products of the traditional ovens and the FTT.

The highest ($p<0.05$) intakes of both smoked-soft and smoked-dry products were reported by Greater Accra Region (Fig. 4.1). This could be attributed to the greater availability of, and higher accessibility to, smoked fish in that area (coastal region) due to the predominance of fishing and fish smoking activities there (Aduomih, 2019). For the smoked-soft product, the Upper East Region recorded the least intake ($p<0.05$). The region is located in the northern part of Ghana where primary production and processing of fishery products is limited (Asiedu et al., 2018). Such areas depend mainly on trade for smoked fish supply. However, due to their short shelf-life (≤ three days under ambient temperature storage) (SNV, 2016), smoked-soft products are traded over a short distribution radius. Consequently, regions further from coastal and inland fisheries communities (such as the Upper East) receive lower supplies of that product. That may account for the least intake reported for the Upper East. For that same reason, it is seen that intakes of the smoked-dry product did not differ ($p>0.05$) between the Upper East and the Brong Ahafo Region, since the availability of that product is enhanced by trade (long shelf-life and wider distribution radius (SNV, 2016)).

Between the two product forms, a higher consumption of smoked-dry *Sardinella* sp. was reported in all the regions (Fig. 4.1, $p<0.05$). Beside their greater availability, the smoked-dry product also has more food applications in the Ghanaian context than the smoked-soft products.

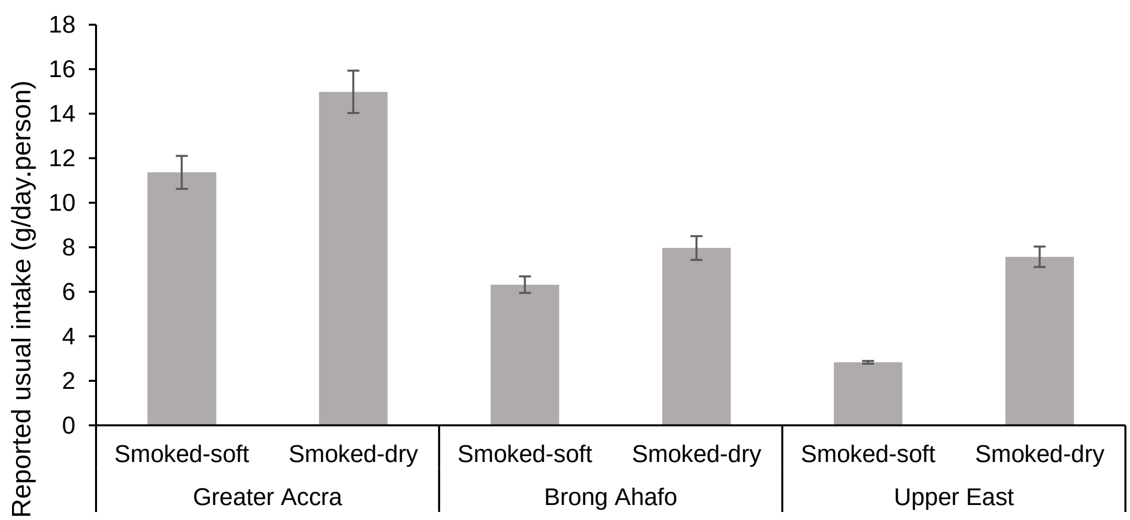

Fig. 4.1: Reported usual intakes (mean g/day.person ±SE) of smoked-soft and smoked-dry *Sardinella* sp. among the study areas. (n=212 for Greater Accra, n=300 each for Brong Ahafor and Upper East)

Best-fit distributions of the consumption data are presented in Table 4.1 (expressed as kg/kg bw/day). These distributions serve to highlight the spectrum of intakes from the lowest to the highest possible amounts, to support probabilistic hazard exposure calculations (Vose, 2008).

4.2.2 PAH levels in products from the markets

As was found for smoked fish from markets in Accra (Chapter 2), the BaP and PAH4 levels in products from the other markets were in the range 16 ± 2 to 63 ± 14 µg/kg and 173 ±11 to 471 ± 18 µg/kg, respectively (Table 4.2). Thus, all the products exceeded the EU limits (2µg/kg for BaP and 12µg/kg for PAH4, Regulation (EC) No. 835/2011). Products from Greater Accra markets were the most contaminated ($p<0.05$) (Table 4.2). For example, PAH4 in smoked-dry *Sardinella* sp. from that region was up to twice the level in Brong Ahafo and Upper East (Table 4.2). As discussed in Chapter 2, this may be explained, particularly in the case of smoked-dried products, by the practice of re-smoking. This is more common in regions (such as the Greater Accra Region) where the volumes of smoked fish produced are high (Ahadzi, 2017).

Table 4.3 shows the best-fit probabilistic distributions for the PAH levels in the smoked products from the markets.

Table 4.1: Best-fit distribution functions for the consumption of smoked-soft and smoked-dry *Sardinella* sp. (kg/kg bw/day) among adults (≥16y) in selected regions of Ghana

Product	Region	Best-fit distribution function	Minimum	Maximum	Mean	P50	P90	P95	P99
Smoked-soft *Sardinella* sp.	Greater Accra	RiskUniform(0.0000200237,0.00108)	2.00E-05	1.08E-03	5.50E-04	5.50E-04	9.74E-04	1.03E-03	1.07E-03
	Brong Ahafo	RiskTriang(0.00005,0.00005,0.0004837)	5.00E-05	4.82E-04	1.95E-04	1.77E-04	3.47E-04	3.87E-04	4.40E-04
	Upper East	RiskUniform(0.0000247492,0.000100251)	2.47E-05	1.00E-04	6.25E-05	6.25E-05	9.27E-05	9.65E-05	9.95E-05
Smoked-dry *Sardinella* sp.	Greater Accra	RiskTriang(0.000025,0.000025,0.0014417)	2.50E-05	1.44E-03	4.97E-04	4.40E-04	9.94E-04	1.12E-03	1.30E-03
	Brong Ahafo	RiskTriang(0.000025,0.000025,0.0014446)	2.50E-05	1.44E-03	4.98E-04	4.41E-04	9.96E-04	1.13E-03	1.30E-03
	Upper East	RiskTriang(0.0000197471,0.00005,0.00049649)	2.02E-05	4.96E-04	1.89E-04	1.70E-04	3.51E-04	3.93E-04	4.50E-04

For smoked-soft herring, trend for intakes was Greater Accra>Brong Ahafo>Upper East ($p<0.05$)
For smoked-dry herring, trend for intake was Greater Accra >Brong Ahafo, Upper East ($p<0.05$) and Brong Ahafo = Upper east ($p>0.05$)

Table 4.2: Mean PAH±stdev (µg/kg) in smoked *Sardinella* sp. from markets in selected regions of Ghana (n=10 per product per region)

Product	Region	BaP	Benzo(a)anthracene	Benzo(b)fluoranthene	Chrysene	PAH4
Smoked-soft *Sardinella* sp.	Greater Accra	48 ± 13[a]	135 ± 33	63 ± 11	165 ± 23	411 ± 66[a]
	Brong Ahafo	26 ± 11[b]	81 ± 27	42 ± 16	128 ± 44	277 ± 97[b]
	Upper East	16 ± 2[c]	50 ± 4	24 ± 2	83 ± 5	173 ± 11[c]
Smoked-dry *Sardinella* sp.	Greater Accra	63 ± 14[a]	149 ± 69	78 ± 24	179 ± 75	471 ± 18[a]
	Brong Ahafo	43 ± 13[b]	79 ± 15	52 ± 17	147 ± 14	311 ± 34[b]
	Upper East	30 ± 18[c]	52 ± 34	37 ± 20	73 ± 51	195 ± 122[c]

Statistical analysis was focused on BaP and PAH4, since those are the regulatory markers for PAHs. For the BaP and PAH4 columns, different superscripts denote significant differences at ($p<0.05$) within each product type.

Table 4.3: Best-fit distributions for PAH levels (µg/kg) in smoked-soft and smoked-dry *Sardinella* sp. from markets in selected regions of Ghana

PAH marker and product	Region	Best-fit distribution function	Minimum	Maximum	Mean†	P50	P90	P95	P99
BaP in smoked-soft *Sardinella* sp.	Greater Accra	RiskTriang(34,34,79.402)	34.0	79.3	49.1	47.3	65.0	69.3	74.9
	Brong Ahafo	RiskBetaGeneral(0.22447,0.22607,14,39)	14.0	39.0	26.5	26.4	38.9	39.0	39.0
	Upper East	RiskTriang(13,13,21.4345)	13.0	21.4	15.8	15.5	18.8	19.6	20.6
PAH4 in smoked-soft *Sardinella* sp.	Greater Accra	RiskTriang(333,333,576.93)	333.0	576.3	414.3	404.4	499.8	522.4	552.5
	Brong Ahafo	RiskUniform(146.22,429.78)	146.2	429.8	288.0	287.9	401.4	415.6	426.9
	Upper East	RiskTriang(143.441,188,188)	143.6	188.0	173.2	174.9	185.7	186.9	187.8
BaP in smoked-dry *Sardinella* sp.	Greater Accra	RiskTriang(42,42,100.863)	42.0	100.6	61.6	59.2	82.3	87.7	94.9
	Brong Ahafo	RiskBetaGeneral(0.19611,0.16113,27,60)	27.0	60.0	45.1	49.1	60.0	60.0	60.0
	Upper East	RiskTriang(12,12,72.694)	12.0	72.4	32.2	29.8	53.5	59.1	66.6
PAH4 in smoked-dry *Sardinella* sp.	Greater Accra	RiskTriang(239,239,958.31)	239.0	955.3	478.8	449.7	730.8	797.5	886.4
	Brong Ahafo	RiskBetaGeneral(0.20215,0.27399,277,382)	277.0	382.0	321.6	307.6	381.6	381.9	382.0
	Upper East	RiskTriang(68,68,479.36)	68.0	478.7	205.1	188.5	349.3	387.4	438.2

†The best-fit probability distributions in this table are for the BaP and PAH4 values in Table 2. As a result, the arithmetic means in Table 2 may differ from the distribution means in this table.

4.2.3 PAH levels in products sampled directly from the FTT and the traditional ovens

The differences in contamination levels among the products of the three ovens (i.e. FTT < metal drum oven < Chorkor smoker) were discussed in Chapters 2 and 3. The probabilistic distributions for the relevant data in those chapters are given in Table 4.4.

4.2.3.1 Effect of removing the skin of smoked fish on PAH level

BaP and PAH4 levels in the descaled smoked-dry *Sardinella* sp. (produced on the Chorkor smoker) were 7.8 ± 0.7 µg/kg and 78 ± 4 µg/kg, respectively (see note π under Table 4.4). The corresponding values for the product bearing the skin were 60 ± 4 µg/kg and 395 ± 17 µg/kg (Chapter 2, Table 2.4). Thus, descaling resulted in 87% and 80% reduction in the BaP and PAH4 levels, respectively. This suggests that the PAH contamination occurred primarily on the upper layers of the products. Duedahl-Olesen et al., (2010) also found that PAH contamination in the skin of smoked fish was over five times higher than in other layers of the product. It may seem, then, that removing the skin of traditionally smoked fish could be a mitigation measure at the consumer end. However, it is noted that the high reduction notwithstanding, the remaining contaminant levels exceeded the EU limits. Additionally, not all smoked (fish) products can be treated this way; for some (such as smoked fish fillets), there may be no skin to remove, while for several others (such as smoked-dry *Silurus* sp. and *Oreochromis* sp.), the skin on the final product may be so tightly bound to the meat layers that removing may not be possible.

In Tanzania, Mahugija and Njale (2018) studied the effect of washing smoked fish in warm (60°C) distilled water on the PAH level in the products and found varying degrees of reduction (31.5% to 86.5%), depending on the fish species. The authors also reported that even with such reductions, the remaining hazard levels in the washed products were above the EU limits (ibid). Furthermore, to some consumers, the sensory appeal of products treated that way may be compromised. Consumer food preparation practices may thus be unreliable as an effective mitigation measure for the elevated hazard levels.

Table 4.4: Best-fit distribution functions for PAH levels (µg/kg) in smoked-dry and smoked-soft *Sardinella* sp. processed separately on the traditional ovens and the FTT (distributions of data from Chapter 3)

PAH marker and product	Oven	Best-fit distribution function	Minimum	Maximum	Mean	P50	P90	P95	P99
BaP in smoked-soft *Sardinella* sp.	Chorkor smoker	RiskBetaGeneral(0.16407,0.16406,18.5,34.3)	18.5	34.3	26.4	26.4	34.3	34.3	34.3
	Metal drum	RiskBetaGeneral(0.16017,0.1474,7.5,13.4)	7.5	13.4	10.6	10.9	13.4	13.4	13.4
	FTT	RiskBetaGeneral(0.057246,0.12807,0.2,0.22)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
PAH4 in smoked-soft *Sardinella* sp.	Chorkor smoker	RiskBetaGeneral(0.15084,0.13054,157.8,172.8)	157.8	172.8	165.9	167.6	172.8	172.8	172.8
	Metal drum	RiskBetaGeneral(0.14672,0.14917,52.52,63.9)	52.5	63.9	58.2	58.0	63.9	63.9	63.9
	FTT	RiskBetaGeneral(0.14703,0.15022,1.37,1.65)	1.4	1.7	1.51	1.5	1.7	1.7	1.7
BaP in smoked-dry *Sardinella* sp.	Chorkor smoker	RiskBetaGeneral(0.16248,0.16237,54.27,66.3)	54.3	66.3	60.3	60.3	66.3	66.3	66.3
	Chorkor smoker$^{\pi}$	RiskTriang(7,7.8,9)	7.0	9.0	7.9	7.9	8.5	8.6	8.8
	Metal drum	RiskBetaGeneral(0.14917,0.14878,23.01,28.12)	23.0	28.1	25.6	25.6	28.1	28.1	28.1
	FTT	RiskTriang(0.2,0.2,0.45429)	0.2	0.5	0.3	0.3	0.4	0.4	0.4
PAH4 in smoked-dry *Sardinella* sp.	Chorkor smoker	RiskBetaGeneral(0.16559,0.16549,369.61,419.37)	369.6	419.4	394.5	394.5	419.4	419.4	419.4
	Chorkor smoker$^{\pi}$	RiskTriang(70,75,90)	70.1	89.9	78.3	77.8	84.5	86.1	83.2
	Metal drum	RiskUniform(115.345,156.055)	115.4	156.1	135.7	135.7	152.0	154.0	155.7
	FTT	RiskBetaGeneral(0.15618,0.16103,1.2,3.3)	1.2	3.3	2.2	2.2	3.3	3.3	3.3

π: The skin of the smoked product was removed prior to determining the PAH contamination level. Arithmetic means for BaP and PAH for this product were 7.8 ± 0.7 µg/kg and 78 ± 4 µg/kg, respectively.

4.2.4 Probabilistic PAH exposure and risk assessment

4.2.4.1 Scenario 1: PAH exposure in products from the markets (reflecting prevailing exposure in traditionally smoked fish in Ghana)

The probabilistic chronic exposure and MoE for PAH in smoked fish from the markets are shown in Table 4.5. Generally, market products accounted for the lowest MoE values (indicating the highest level of public health concern). For example, the mean BaP MoE in smoked-soft *Sardinella* sp. in Greater Accra was 3,700 whereas the corresponding value in the Chorkor smoker product was 6,892 (Table 4.6). Greater Accra Region recorded the highest exposure whereas those in the Upper East Region recorded the lowest. The mean BaP and PAH4 exposure in smoked-soft products in Greater Accra Region were respectively 25- and 5-times the exposure in the Upper East Region (Table 4.5). The PAH contamination (Table 4.2) and fish intake (Fig. 4.1 and Table 4.1) were also highest in Greater Accra Region and lowest in the Upper East Region ($p<0.05$). High hazard concentrations in a food commodity coupled with high intakes of that food results in high hazard exposure, hence the observations. Across the regions, exposure was generally lower for smoked-soft *Sardinella* sp. owing to the lower concentration of the hazard in such products (Table 4.2, $p<0.05$) and lower consumption of same.

The MoE values in Table 4.5 suggest a public health concern of high PAH exposure in smoked fish in Ghana. For example, at all calculated percentiles, MoE (for both BaP and PAH4) for Greater Accra were less than 10,000. Similarly, with the exception of the smoked-soft product from the Upper East region, all P90 values of the MoE for all other products were less than 10,000 across the regions. As noted earlier, for PAHs, an MoE less than 10,000 is considered to denote a potential public health problem requiring risk management intervention (EFSA, 2005). Since smoked fish on markets are invariably processed on traditional ovens (i.e. either Chorkor smoker or metal drum oven), the MoE values point to potential PAH-related food safety and public health problems linked to the use of those ovens.

Essumang et al. (2012) also reported a similarly high PAH exposure in smoked *Sardinella* sp. in Ghana and recommended improvements in smoking techniques as an intervention.

Table 4.5: Probabilistic chronic exposure (µg/kg bw day) and corresponding margins of exposure (MoE) for PAH in smoked *Sardinella* sp. from markets in selected regions of Ghana (Scenario 1: Prevailing situation of PAH exposure in smoked fish on markets)

	PAH marker and product	**Region of market**	**Measure**	**Minimum**	**Maximum**	**Mean**	**P50**	**P90**	**P95**	**P99**
Smoked-soft *Sardinella* sp.	BaP in smoked-soft *Sardinella* sp.	Greater Accra	Exposure	7.09E-04	8.37E-02	2.70E-02	2.58E-02	4.96E-02	5.65E-02	6.88E-02
			MoE	141,112	**1,194**	**3,700**	**3,873**	**2,017**	**1,770**	**1,454**
		Brong Ahafo	Exposure	7.02E-04	1.85E-02	5.15E-03	4.17E-03	1.05E-02	1.26E-02	1.58E-02
			MoE	142,454	**5,406**	19,431	23,986	**9,552**	**7,923**	**6,327**
		Upper East	Exposure	3.25E-04	2.10E-03	9.88E-04	9.74E-04	1.49E-03	1.62E-03	1.83E-03
			MoE	308,059	47,608	101,203	102,695	67,313	61,873	54,794
	PAH4 in smoked-soft *Sardinella* sp.	Greater Accra	Exposure	6.89E-03	6.07E-01	2.28E-01	2.24E-01	4.06E-01	4.49E-01	5.20E-01
			MoE	49,314	**560**	**1,491**	**1,521**	**837**	**758**	**654**
		Brong Ahafo	Exposure	7.38E-03	2.02E-01	5.61E-02	4.79E-02	1.07E-01	1.25E-01	1.58E-01
			MoE	46,074	**1,680**	**6,064**	**7,092**	**3,188**	**2,717**	**2,159**
		Upper East	Exposure	3.63E-03	1.88E-02	1.08E-02	1.08E-02	1.61E-02	1.70E-02	1.80E-02
			MoE	93,570	18,050	31,414	31,549	21,135	20,049	18,855
Smoked-dry *Sardinella* sp.	BaP in smoked-dry *Sardinella* sp.	Greater Accra	Exposure	1.06E-03	1.33E-01	3.06E-02	2.61E-02	6.19E-02	7.34E-02	9.49E-02
			MoE	94,132	**752**	**3,264**	**3,832**	**1,614**	**1,363**	**1,054**
		Brong Ahafo	Exposure	6.76E-04	8.57E-02	2.25E-02	1.83E-02	4.83E-02	5.86E-02	7.27E-02
			MoE	147,858	**1,167**	**4,447**	**5,479**	**2,071**	**1,705**	**1,375**
		Upper East	Exposure	2.65E-04	3.42E-02	6.09E-03	4.75E-03	1.27E-02	1.58E-02	2.16E-02
			MoE	378,037	**2,927**	16,422	21,054	**7,853**	**6,332**	**4,631**
	PAH4 in smoked-dry *Sardinella* sp.	Greater Accra	Exposure	6.31E-03	1.31E+00	2.38E-01	1.90E-01	5.04E-01	6.20E-01	8.52E-01
			MoE	53,851	**260**	**1,429**	**1,788**	**675**	**549**	**399**
		Brong Ahafo	Exposure	6.93E-03	5.49E-01	1.60E-01	1.40E-01	3.21E-01	3.69E-01	4.54E-01
			MoE	49,048	**619**	**2,121**	**2,430**	**1,058**	**921**	**749**
		Upper East	Exposure	1.50E-03	2.18E-01	3.87E-02	2.96E-02	8.12E-02	1.02E-01	1.42E-01
			MoE	226,929	**1,563**	**8,797**	11,476	**4,186**	**3,325**	**2,396**

Note: MoE less than 10,000 are presented in **bold font** and suggest a public health concern requiring an intervention (EFSA, 2005).

4.2.4.2 Scenario 2: Impact of smoking technique on PAH exposure (Traditional ovens vs. the FTT)

Among the three ovens, PAH exposure was highest in the Chorkor smoker products and lowest in FTT products ($p<0.05$) (Table 4.6), following the same pattern as PAH levels in products from the ovens. Mean BaP exposure in smoked-soft *Sardinella* sp. from the FTT was 128 and 52 times, respectively, lower than the exposure in products from the Chorkor smoker and metal drum oven (Table 4.6). Furthermore, at all considered percentiles, the MoE for BaP and PAH4 for both smoked-soft and smoked-dry products was in the order Chorkor smoker < metal drum oven < FTT (Table 4.6). For example, at P90, the MoE for PAH4 in smoked-dry *Sardinella* sp. was 865 for the Chorkor smoker; 2,514 for the metal drum oven and 134,432 for the FTT (Table 4.6). These values show, on the one hand, the extent of the PAH food safety problem with traditional oven products and, on the other hand, the positive impact of the FTT as an intervention (in increasing the MoE far above 10,000).

Considering that the metal drum products presented a lower exposure than the Chorkor smoker products, it is significant to recall that the latter was developed in Ghana in 1969 as an improved oven to address primarily the low fuel efficiency and high operational drudgery associated with the former (Brownell, 1983; Chapter 1). The findings of the present study points to the need for including risk-based food safety considerations in introducing new technologies in food processing, since the food safety problem (PAH exposure) was higher in Chorkor smoker products than in those from the metal drum oven.

High PAH levels in smoked fish is an acknowledged problem in regions of the world where traditional hot-smoking is practiced (especially in Africa and Asia). The International Association of Fish Inspectors (IAFI) has noted that in West Africa, the average PAH exposure from smoked fish alone is about ten times the PAH exposure from all food sources in the EU (IAFI, 2017). The Association has, therefore, called on governments in developing countries to prioritize the reduction of PAH health risks linked to the consumption of smoked fish (IAFI, 2017)

Table 4.6: Probabilistic chronic exposure (µg/kg bw/day) and corresponding margins of exposure (MoE) for PAHs in *Sardinella* sp. smoked separately on the traditional ovens and the FTT (Representing Exposure Scenario 2: Traditional ovens vs. FTT & Scenario 3: Effect of removing the skin of smoked products)

	PAH marker and product	Oven	Measure	Minimum	Maximum	Mean	P50	P90	P95	P99
Smoked-soft *Sardinella* sp.	BaP in smoked-soft *Sardinella* sp.	Chorkor smoker	Exposure	3.73E-04	3.70E-02	1.45E-02	1.35E-02	2.84E-02	3.21E-02	3.58E-02
			MoE	268,003	**2,700**	**6,892**	**7,397**	**3,521**	**3,116**	**2,795**
		Metal Drum	Exposure	1.51E-04	1.45E-02	5.82E-03	5.46E-03	1.13E-02	1.27E-02	1.40E-02
			MoE	661,738	**6,910**	17,181	18,305	**8,835**	**7,885**	**7,131**
		FTT	Exposure	4.01E-06	2.38E-04	1.13E-04	1.13E-04	2.00E-04	2.11E-04	2.28E-04
			MoE	24,966,775	421,035	881,807	882,963	498,869	473,175	438,114
	PAH4 in smoked-soft *Sardinella* sp.	Chorkor smoker	Exposure	3.16E-03	1.87E-01	9.12E-02	9.11E-02	1.61E-01	1.70E-01	1.82E-01
			MoE	107,431	**1,822**	**3,728**	**3,733**	**2,109**	**1,999**	**1,866**
		Metal Drum	Exposure	1.05E-03	6.90E-02	3.20E-02	3.18E-02	5.62E-02	6.14E-02	6.71E-02
			MoE	322,784	**4,927**	10,630	10,690	**6,048**	**5,542**	**5,069**
		FTT	Exposure	2.74E-05	1.78E-03	8.30E-04	8.25E-04	1.46E-03	1.59E-03	1.73E-03
			MoE	12,392,224	190,818	409,632	412,160	232,775	214,446	196,211
Smoked-dry *Sardinella* sp.	BaP in smoked-dry *Sardinella* sp.	Chorkor smoker	Exposure	1.36E-03	9.44E-02	3.00E-02	2.63E-02	6.00E-02	6.84E-02	8.10E-02
			MoE	73,615	**1,060**	**3,336**	**3,800**	**1,667**	**1,463**	**1,234**
		Chorkor smoker (skin removed)[π]	Exposure	1.80E-04	9.28E-03	3.04E-03	2.68E-03	6.06E-03	6.85E-03	7.92E-03
			MoE	556,538	10,771	32,873	37,292	16,499	14,598	12,634
		Metal Drum	Exposure	5.78E-04	4.00E-02	1.27E-02	1.12E-02	2.54E-02	2.90E-02	3.45E-02
			MoE	173,151	**2,497**	**7,863**	**8,954**	**3,937**	**3,449**	**2,897**
		FTT	Exposure	5.07E-06	6.21E-04	1.42E-04	1.21E-04	2.86E-04	3.38E-04	4.33E-04
			MoE	19,735,957	160,955	705,592	827,610	349,832	295,985	230,825
	PAH4 in smoked-dry *Sardinella* sp.	Chorkor smoker	Exposure	9.29E-03	5.94E-01	1.96E-01	1.73E-01	3.93E-01	4.45E-01	5.18E-01
			MoE	36,597	**572**	**1,733**	**1,963**	**865**	**764**	**657**
		Chorkor smoker (skin removed)[π]	Exposure	1.87E-03	9.09E-02	3.00E-02	2.66E-02	5.98E-02	6.79E-02	7.80E-02
			MoE	18,1340	**3,739**	11,324	12,781	**5,684**	**5,007**	**4,358**
		Metal Drum	Exposure	2.89E-03	2.20E-01	6.75E-02	5.93E-02	1.35E-01	1.54E-01	1.82E-01
			MoE	117,643	**1,544**	**5,039**	**5,729**	**2,514**	**2,210**	**1,869**
		FTT	Exposure	3.00E-05	4.71E-03	1.11E-03	8.49E-04	2.53E-03	3.11E-03	3.93E-03
			MoE	11,325,534	72,238	305,759	400,439	134,432	109,470	86,540

Note: MoE less than 10,000 are presented in **bold font** and denote a PAH public health concern associated with the product (EFSA, 2005). Note that none of the products from the FTT presents such a concern (i.e. all MoE values for FTT products >10,000)

π: The skin of the smoked product was removed prior to determining the PAH contamination level (Scenario 3)

4.2.4.3 Scenario 3: Impact of removing the skin of traditionally smoked fish on exposure to PAH

The reduction in PAH levels due to descaling of smoked-dry *Sardinella* sp. from the Chorkor smoker (Table 4.4) translated into reduced exposure for BaP (Table 4.6). For that product, BaP MoE at all percentiles were above the 10,000 threshold, indicating a low public health concern. However, for the same product, the P90, P95, P99 and maximum MoE of PAH4 were all below 10,000 (Table 4.6). For public health protection, low exposure is required for *both* BaP and PAH4. Therefore, the treatment may not be considered a sustainable risk mitigation strategy, since it does not reduce overall PAH exposure to an acceptable level. A sustainable approach could be to prevent the elevated contamination at the processing step, rather than attempting to reduce it after the fact.

Furthermore, removing the skin of smoked fish may also compromise the overall nutritional value of the product. Rebolé et al. (2015) reported higher amounts of calcium (eight-times higher), iron (two-times higher) manganese (five-times higher) and zinc (three-times higher) in the skin of rainbow trout than in the muscle. Average lipid content has also been reported to be higher (over 60%) in fish skin than in the muscles (Ortiz et al., 2013; Rebolé et al., 2015). In addition, the loss of lipids in the skin may also result in loss of fat-soluble vitamins. Thus, removing the skin of smoked fish reduces the availability of these micro- and macronutrients to consumers.

Given that smoking with traditional ovens is the predominant method for fish preservation in Ghana (Nketsia-Tabiri and Sefa-Dedeh, 2000; Nerquaye-Tetteh et al., 2002; Nti et al., 2002), that most fish species and other sources of animal protein (such as meat) are smoked, and that traditional oven products have high PAH levels, it follows that, in a total diet study for PAHs, the exposure to the hazard in all smoked foods in the country may be much higher than the exposure reported for only smoked *Sardinella* sp. in this study. Hence, the public health problem of using traditional ovens may be much bigger when other smoked foods are considered, and the impact of the FTT as an intervention that much greater.

The limitations identified for the probabilistic exposure assessment are that only the adult (≥16 years old) population was considered, and a body weight of 60 kg was assumed for all respondents. These, however, do not detract from the observed high PAH exposure associated with smoked fish from the traditional ovens (whether sourced from the markets or directly from processing sites) and the potency of the FTT as a potential intervention.

4.3 CONCLUSION

The findings suggest that the use of traditional ovens for fish smoking results in high consumer exposure to PAHs in smoked fish in Ghana, and that the FTT is a viable technical intervention for reducing the exposure. Removing the skin of traditionally smoked fish reduces the level of PAHs but does not necessarily reduce the consumer exposure to the hazard. For risk management, it may be more efficient to prevent the excessive contamination of smoked fish through the use of improved ovens (such as the FTT), than relying on consumer cooking practices to reduce the contamination post-processing.

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Although, as has been shown, the FTT could reduce human exposure to PAH health risks in smoked fish, will the sensory attributes of its products be accepted by the Ghanaian consumer? This was the interest of specific objective 4 and is discussed in Chapter 5.

CHAPTER 5: EVALUATION OF CONSUMER ACCEPTANCE OF FTT PRODUCTS IN GHANA

ABSTRACT

To measure consumer acceptance of FTT product attributes, smoked-soft mackerel (*Scomber japonicus*) produced on that oven and the Chorkor smoker were evaluated for their sensory profiles and consumer responses to same. Using the quantitative descriptive analysis (QDA) method, a panel of 14 assessors were trained to describe and score the appearance, aroma, taste, texture, mouthfeel and aftereffect of products from the two ovens. Subsequently, in light of the QDA results, 90 untrained panellists (Ghanaian consumers) evaluated the product attributes on a nine-point hedonic scale by assigning scores from 1 (dislike extremely) to 9 (like extremely). For the QDA, attributes with significant differences ($p < 0.05$) were appearance (Chorkor smoker products darker with score 7.1 ± 0.6 vs. 4.9 ± 0.6 for FTT, $p=0.004$), texture (FTT products drier with score 10.6 ± 0.6 vs. 8.6 ± 0.6 for the Chorkor smoker, $p=0.035$) and mouthfeel (FTT products drier with score 8.6 ± 0.7 vs. 6.9 ± 0.7 for the Chorkor smoker, $p=0.030$). These differences notwithstanding, in the consumer acceptance test, no significant differences ($p>0.05$) were found in any of the attributes of the products from the two ovens. The findings suggest that although there are perceptible sensorial differences between FTT and Chorkor smoker products, they did not result in consumer preference of one product over the other. Therefore, consumers are likely to accept FTT product attributes just as much as they do the Chorkor smoker products.

Keywords: consumer acceptance, smoked fish, smoking oven

5.0 INTRODUCTION

It the preceding chapters, the safety of FTT products was evaluated (Chapter 3) and the impact of such safety on public health protection demonstrated (Chapter 4). In this chapter, the response of Ghanaian consumers to the sensory attributes of FTT products is assessed.

In (new) food product and process development, determining consumer response to sensory quality cues is an important step for confirming whether consumers are/will be adequately satisfied with characteristics that are either intentionally engineered into foods or that (could) result from the processing technique applied (Pecore and Kellen, 2002; O'Sullivan et al., 2011). This is crucial since without consumer patronage, neither the economic nor food and nutrition security benefits of food processing/preservation can be attained. In the case of the introduction of the FTT, this task is doubly important because both the product (smoked fish) and the process (improved smoking oven) have novel aspects that must be acceptable to their respective end-users.

The evaluation of consumer response to food is done through sensory analysis; the scientific discipline used to evoke, measure, analyse, and interpret human reactions to the characteristics of foods as they are perceived by the senses of sight, smell, touch, taste and hearing (Stone and Sidel, 2003). It relies on the use of humans as instruments to identify and measure the intensities of perceptible food characteristics (Lawless and Heymann, 2010; Stone et al., 2012). Three broad approaches are used, namely difference test, descriptive quantitative analysis and affective test (Pecore and Kellen, 2002; O'Sullivan et al., 2011).

Difference tests determine if assessors can tell one product apart from the other (Byrne and Bredie, 2002). As sensory responses to the same product can vary even for the same person (Michon et al., 2010), assessors can be trained and calibrated to quantitatively and objectively measure sensory modalities (such as appearance, texture, taste and aftereffect) and give standard responses (Yusop et al., 2009). This is called a descriptive quantitative test (Lawless and Heymann, 2010). It does not accommodate the expression of personal liking of assessors for the attributes (O'Sullivan et al., 2011). Affective tests require assessors (typically untrained) to state how much they like the sensory modalities of a product (O'Sullivan et al., 2011). Consumer acceptance test is an example of an affective test. It is subjective in nature since respondents are asked to state their feeling towards a product using anchors (words describing intensities such as none, low, medium or high) (Bryhni et al., 2002).

For the food safety and public health benefits of the FTT to be realised domestically, the Ghanaian consumer should be willing to patronize its products. Among other factors, determining how far this is the case is important for establishing a warrant for the adoption and use of the oven. To provide evidence in that regard, this study evaluated the sensory profile of smoked fish from the FTT and the Chorkor smoker through a quantitative descriptive test, and also determined whether consumers – unaware of the history and added food safety value of FTT products – could show varying degrees of liking between products from the two ovens.

5.1 MATERIALS AND METHODS

Chub mackerel (*Scomber japonicus*, hereafter called mackerel) was smoked-soft on the FTT and the Chorkor smoker separately, according to the process flow described in Chapter 1 (Fig. 1.9) and under the observation of the researcher. *Sardinella* sp. was not used for the sensory evaluation due its bony nature that would have made handling during the testing process cumbersome. Mackerel, on the hand, is much less bony and thus easier to handle. Two tests were conducted in sequence to determine the sensory profiles of the products and consumer responses to same. Firstly, a quantitative descriptive analysis (QDA) was used to highlight the sensory attributes of the products from the two ovens and determine the degree to which they differed in intensity. Secondly, a consumer acceptance test was used to compare how much consumers liked the attributes of the products from the two ovens. Both tests were conducted in Ghana in the Sensory Science Laboratory of the Department of Nutrition and Food Science, University of Ghana (UG).

5.1.1 Quantitative descriptive analysis (QDA) protocol

For the QDA, a 14-member trained panel (assessors experienced in sensory evaluation in general, and affiliated with the UG Sensory Science Laboratory) were re-trained to specifically evaluate smoked mackerel on six sensory modalities, namely appearance, aroma, texture, taste, mouthfeel and aftereffects. These attributes were selected as they constitute the major properties of food materials considered in sensory analysis (Lee et al., 2008). Mouthfeel refers to the oral tactile sensations arising from the interaction between ingested food and the receptors in the mouth during chewing (Lawless and Heymann, 2010). Aftereffects are the sensations that linger in the mouth after ingested food is either swallowed or spat out (Neely and Borg, 1999).

5.1.1.1 Panel training

The 14-member panel was trained through a series of activities as explained below.

- *Activity 1: Terms generation* – Smoked-soft mackerel was purchased from the Madina market in Accra (this market was selected based on its proximity to the sensory laboratory) for the panel training. The tail and head portions of the products were severed and the remaining section was cut transversely in two portions, each of which was split along the backbone into two halves and de-boned. Portions (~90g per product per serving) were presented to each member of the panel to elicit their individual descriptions of the product based on the six aforementioned sensory modalities. The objective was to identify the words each assessor would use to describe the product

- *Activity 2*: *Consensus building* – The terms used by the individual panellists for each of the sensory modalities were collated. A focus group discussion was then held with the panel to arrive at a uniform definition of all the terms applied to each sensory attribute

- *Activity 3*: *Referencing* – In this activity, discussions were held to identify and select reference materials for the sensory attributes of traditionally smoked fish. Food and non-food materials suggested by the panel were used to further explain the terms to ensure they were used in the same way by all assessors. This is critical to obtaining reliable sensory analysis results (Andani et al., 2001). The following are the references selected and used for the sensory attributes:

- *Burnt notes (appearance, aroma, taste):* Smoked-soft mackerel from the market was heated in an electric oven at 250^{o}C for 15 minutes to char it. The appearance, aroma and taste of this charred product were used as reference for the test products.

- *Traditional product notes (aroma, taste):* Smoked-soft mackerel from the market was used as reference

- *Bitter notes (taste*): The references used were caffeine solution (0.35 g/L, prepared by dissolving 0.087 g in 250 mL of potable water), and quinine tonic water. Bitter notes were also picked in the charred smoked fish

- *Sour notes (taste):* Heinz white vinegar and citric acid solution (0.3 g/L, prepared by dissolving 0.075 g citric acid in 250 mL of potable water) were used as references

- *Smoky notes*: The references used were ham (Bauwens Superior DD Ham) and dried palm kernel mesocarp fibre that was moistened, lit to generate smoke and then smothered to prevent complete burning.

- *Activity 4: Finalizing list of terms for attributes* - A check-all-that-apply (CATA) approach was used to finalize the list of attributes for the modalities by separately evaluating smoked-soft mackerel purchased from the market and test products from the ovens. In CATA assessments, respondents choose from a list terms that apply to the product being evaluated (Ares et al., 2015). For each modality, only attribute terms used consistently by ≥ 50% of the panel (Stone and Sidel, 2003) were included in the final list (Table 5.1).

Table 5.1: Definitions of descriptors used for the sensory evaluation of smoked-soft mackerel (continued on next page)

Modality	Attribute	Definition	Anchors
Appearance	**Dark**	Darkness of the surface (skin) of the smoked product	Low (pale colour) – High (dark colour)
	Oily	Oily skin/surface of product	Low (very little oil on surface) – High (very oily on the surface)
	Burnt	Skin/exterior part of fish appearing charred from excessive heat exposure	Low (very low incidence of charring) – High (very charred product)
	Stale	Exterior of fish looking stale, suggesting product is not freshly produced	Low (low in staleness) – High (very stale)
	Lacerated	Skin/surface of product appearing bruised	Low (very few bruises, if any) – High (very bruised)
	Wrinkled	Skin of product appearing to have folded and detaching from fish meat	Low (firm skin) – High (very wrinkled skin)
	Iridescent	Golden-brown sheen of traditionally smoked mackerel	Low (low intensity of golden-brown colour) – High (intense golden-brown colour)
Aroma	**Smoky**	Characteristic smell of the smoke of dried palm kernel fibre	Low (less smoky) - High (very smoky)
	Traditional aroma	Characteristic aroma of traditionally smoked mackerel	Low (very low intensity) – High (very high intensity)
	Burnt	Aroma reminiscent of burnt oily fish	Low (very low intensity) – High (very high intensity)
Texture	**Dry**	Product feeling dry to the touch	Low (moist) – High (very dry)
	Soft	Easily compressed between the fingers	Low (hard) – High (soft)
	Oily	Oily to the touch	Low (less oily to the touch) – High (very oily to the tough)
	Flaky	Fish meat easily breaks into flakes	Low (less flaky) – High (very flaky)
Taste	**Traditional taste**	Characteristic flavour of traditionally smoked mackerel	Low (low intensity of traditional taste) – High (high intensity of traditional taste)
	Salty	Basic salty taste	Low (very low salty taste) – High (very salty)
	Umami	Basic umami taste	Low (low intensity of umami taste) – High (high intensity of umami taste)
	Smoky	Taste resembling characteristic flavour of the smoke of dried palm kernel fibre	Low (very low in smoky flavour) – High (very high in smoky flavour)
	Burnt	Characteristic taste of charred oily fish	Low (very low in charred taste) – High (very high in charred taste)
	Bitter	Basic bitter taste	Low (very low in bitterness) – High (very bitter)
Mouth feel	**Soft**	Product feels soft against the palate and is easily masticated	Low (very hard against the palate) – High (very soft against the palate)
	Chewy	Product feels dry and chewy during mastication	Low (very low chewiness) – High (very chewy)
	Fibrous	Forms a fibrous mass in the mouth	Low (does not form fibrous mass) – High (forms excessive fibrous mass)

Table 5.1 Definitions of descriptors used for the sensory evaluation of smoked-soft mackerel (continued)

Modality	Attribute	Definition	Anchors
Aftereffect	**Umami**	Umami aftertaste	Low (very low intensity of umami taste in the mouth after swallowing) – High (very intense umami taste in the mouth after swallowing)
	Salty	Salty aftertaste	Low (very low salty aftertaste) – High (very salty aftertaste)
	Smoky	Lingering smoky taste reminiscent of burnt dried palm kernel fibre	Low (very low smoky aftereffect) – High (very intense smoky aftereffect)
	Bitter	Bitter aftertaste	Low (very low biter aftereffect) – High (very intense bitter aftereffect)
	Food trap	Pieces of fish stuck between teeth after chewing and swallowing	Low (very low incidence of food trap) – High (very high incidence of food trap)

Attributes in bold were used in the evaluation. Attributes in normal font were not used.

5.1.1.2 Test sample preparation and serving

Samples were prepared as described in Section 5.1.1.1, randomly coded and served to assessors in a randomised balanced order using the Williams' Design in Compusense Cloud® service-as-a-software (SaaS) (Compusense, Guelph, Ontario, Canada). Products were served on white disposable plates (Fig. 5.1), with disposable fork, knife and tissue. Water and bland crackers were provided for palate cleansing between tastings (Bongoni, 2014).

Fig. 5.1: Servings of smoked-soft mackerel from the FTT (left) and the Chorkor smoker (right)

5.1.1.3 QDA attribute scoring

The intensities of individual attributes were scored on a 15cm line scale anchored at 0 cm for "low intensity" up to 15 cm for "high intensity" on the Compusense Cloud® software (Compusense, Guelph, Ontario, Canada). Samples were evaluated in triplicate per assessor, in a monadic sequential order.

5.1.2 Consumer acceptance test

Ninety (90) apparently healthy Ghanaian adults untrained in sensory evaluation volunteered for the consumer acceptance test by responding to an advertisement in the University of Ghana and completing a recruitment questionnaire. The selection criteria were willingness to participate, non-allergenicity to seafood and that one be a consumer of smoked mackerel. Sample preparation followed the same procedure used for the QDA.

For each product, respondents scored the respective attributes of appearance, texture, flavour and aftertaste, as well as their overall liking, on a nine-point hedonic scale with scores of 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = live very much, 9 = like extremely. In addition, consumers were also asked to indicate which factor (among appearance, taste, texture and price) they considered to be most important when choosing a smoked fish product.

5.1.3 Environmental controls during the sensory tests

For all tests (QDA and consumer acceptance test), assessors were seated in individually partitioned booths in a dedicated, well-lit (white fluorescent light) and airconditioned (temperature 22^{o}C) tasting room of the Sensory Laboratory. The laboratory is equipped with extractor fans to minimize odour contamination. Samples were served through hatches in a partition for the sample preparation room and the testing room. Pre- and post-test discussions with panellists, where required, were held in a dedicated discussion room separated from the tasting and sample preparation rooms. The same environmental controls were available in all the rooms.

5.1.4 Statistical analysis

Data were analysed using SPSS Version 21 (IBM Corp., Armonk, NY, USA). Data were first explored for normality by the Shapiro-Wilk test and the appropriate tests applied to compare means. When comparing means of an attribute between the two ovens (representing two independent samples), normally distributed data were compared by t-tests and non-normally distributed data by the Mann-Whitney U non-parametric test. In cases where, for the same attribute, the data of one oven was normally distributed while the other was not, the non-normal data were first transformed using the square root, log normal or inverse distribution function of SPSS (as appropriate), followed by independent sample t-tests. Significance was set at $p<0.05$.

5.2 RESULTS AND DISCUSSION

In general, in the QDA, perceptible differences were found in some of the sensory properties of smoked-soft mackerel from the FTT and the Chorkor smoker. Of the six modalities assessed, significant differences ($p<0.05$) were found in three (appearance, texture and mouthfeel). No significant differences were found in taste, aroma and aftereffects ($p>0.05$) (Fig. 5.2 – 5.5). In the consumer acceptance test, no significant differences were found in any of the attributes for products of the two ovens ($p>0.05$). The comparisons between the specific attributes of the various modalities and their associated p-values are discussed next.

5.2.1 Quantitative descriptive analysis: FTT product vs. Chorkor smoker product

5.2.1.1 Product appearance

Of the four attributes for appearance (dark, burnt, lacerated and wrinkled, Table 5.1), three were found to differ significantly between the products of the two ovens (Fig. 5.2). The Chorkor smoker product was darker ($p=0.004$) and more wrinkled ($p=0.032$), whereas the FTT product was more lacerated ($p=0.030$). The darker colour of the Chorkor smoker product could be due to higher smoke deposition on the product (discussed in Chapter 3). The wrinkled appearance arose from the looseness of the skin of product, and the lacerated attribute from the dryness of the FTT product to the touch (discussed in a subsequent section (5.2.1.3)). No differences were found in the burnt attribute (p=0.788), as, in fact, neither of the products was charred.

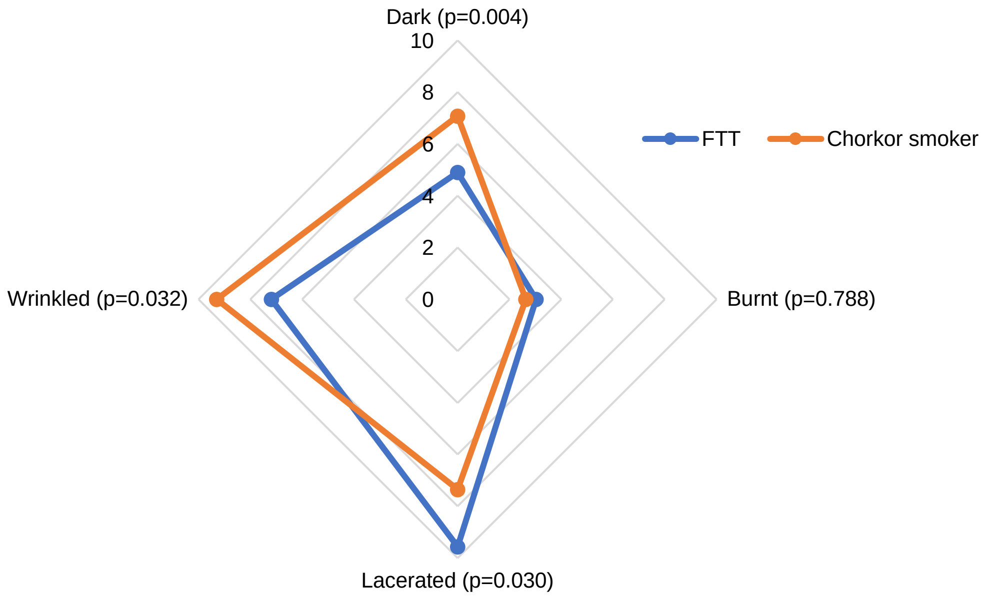

Fig. 5.2: Appearance profile of smoked-soft mackerel (*Scomber japonicus*) produced on the FTT vs. Chorkor smoker (mean attribute scores plotted + p-value)

5.2.1.2 Aroma and taste

Three attributes each were identified and applied for aroma (smoky, burnt, traditional) and taste (smoky, umami, burnt). These attributes are defined in Table 5.1. No significant differences were found on those points between the products (Fig. 5.3). It is noteworthy that for each product, the highest ($p<0.05$) aroma and taste attribute scores were assigned to "traditional" (Fig. 5.3), suggesting that products of both ovens delivered the expected aroma and taste of Ghanaian smoked mackerel. Umami, known to be a characteristic taste attribute in fish products (Komata, 1990; Ninomiya, 2002; Sarower et al., 2012) was found to be the second dominant taste attribute in both products (Fig. 5.3).

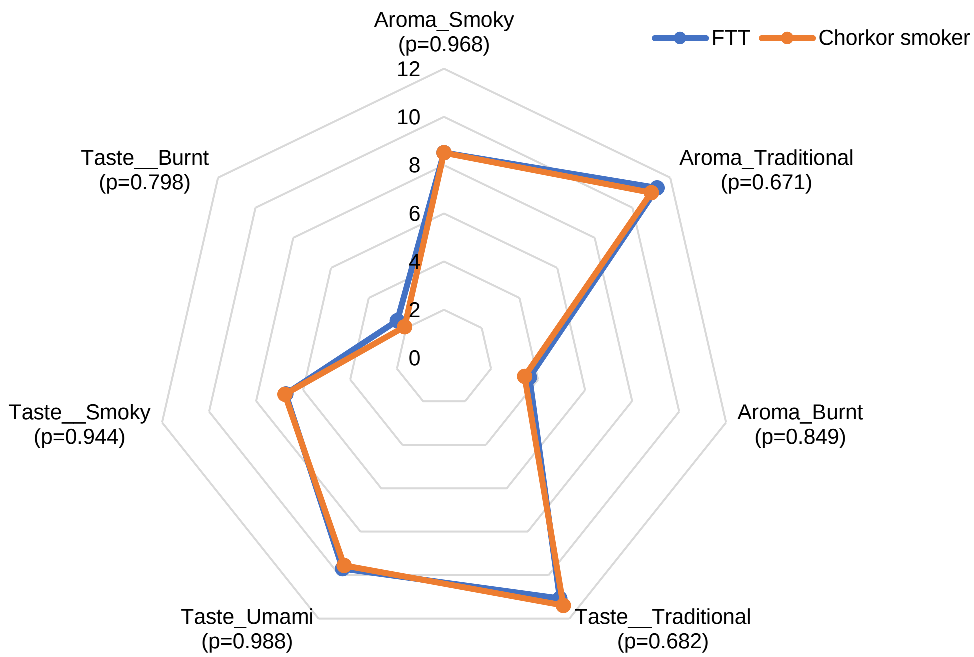

Fig. 5.3: Aroma and taste attribute scores for smoked-soft mackerel (*Scomber japonicus*) produced on the FTT vs. the Chorkor smoker (mean attribute scores plotted + p-vale)

5.2.1.3 Texture and mouthfeel

As defined in Table 5.1, the texture measured the physical feel of the product between the fingers, whereas the mouthfeel measured the physical feel of the product in the mouth. For texture, the FTT product was dryer to the touch (p=0.035) and harder between fingers (p=0.008) and had a chewier (p=0.030) mouthfeel than the Chorkor smoker product. (Fig. 5.4). It is probable that temperature differences during processing may have affected the overall texture of the products. However, since this was not measured during the smoking sessions, a definitive link cannot be established at present. Further, the observed lacerated and wrinkled attributes (Fig. 5.2) of the FTT product may also be partly linked to heat exposure differences. No differences were found between the oil (p=0.753) and flaky (p=0.076) attributes (Fig. 5.4).

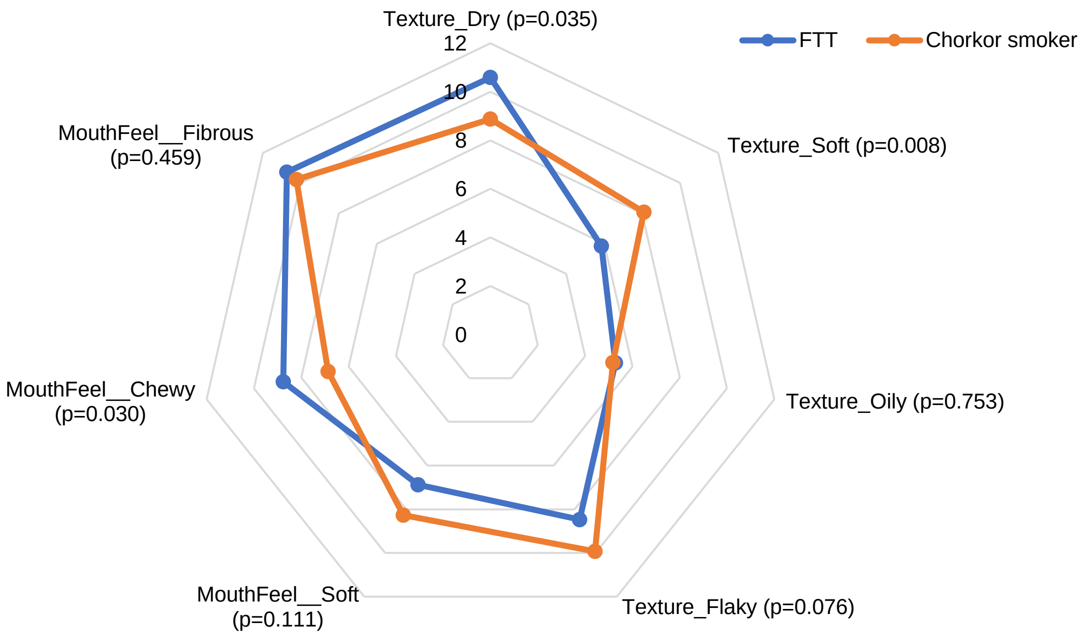

Fig. 5.4: Texture and mouthfeel profile of smoked-soft mackerel (*Scomber japonicus*) produced on the FTT vs. Chorkor smoker (mean attribute scores + p-value)

5.2.1.4 Aftereffects

These tests measured the sensory effect that lingered in the mouth after each product was chewed and swallowed (Neely and Borg, 1999). No significant differences were found for any of the attributes (Fig. 5.5). However, it is seen that the umami taste outlived the traditional smoked fish taste, being felt as the most intense ($p<0.05$) note when the products were swallowed. For both ovens, it is seen that the umami taste recorded the highest intensity ($p<0.05$) (Fig. 5.5).

The food trap (defined in Table 5.1) aftereffect may be explained by the fact that the product was consumed as-is during the test. Typically, smoked fish is consumed in a soup or a sauce, in which medium the product becomes sufficiently moistened or lubricated, enhancing its fineness during mastication and thus reducing the incidence of food trap.

This was not the case in the sensory test; hence the observed food trap aftereffect was not unusual.

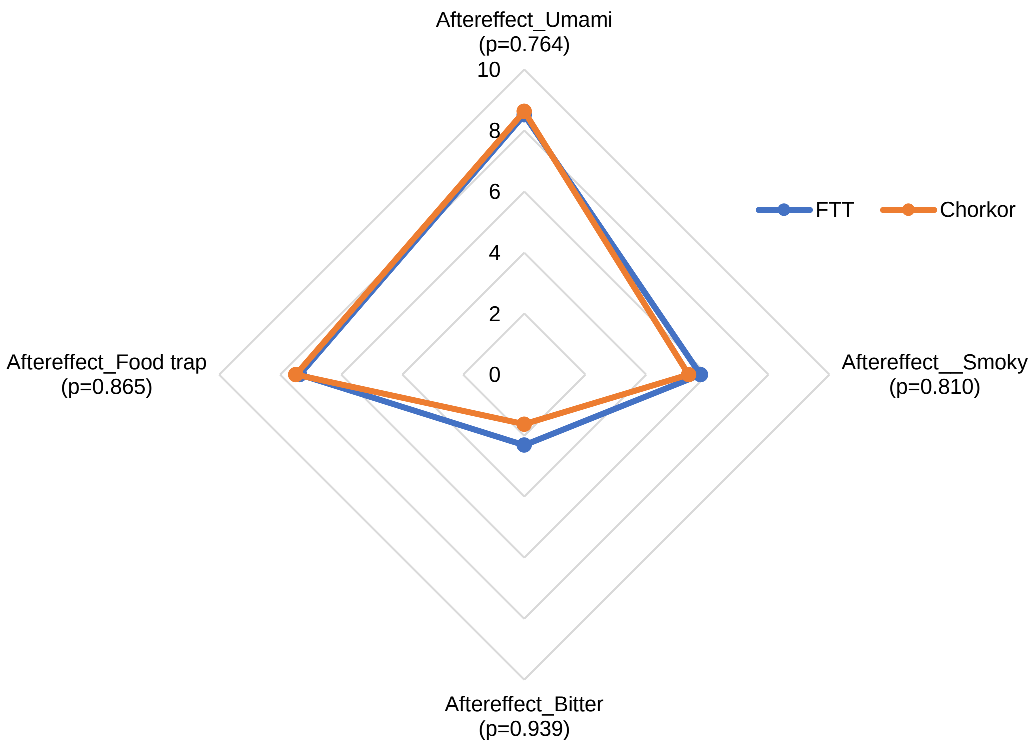

Fig. 5.5: Aftertaste profile of smoked-soft mackerel (*Scomber japonicus*) produced on the FTT vs. Chorkor smoker (mean attribute scores + p-value)

Although assigned the lowest intensity scores, the bitterness aftereffect was nevertheless perceptible in both products (Fig. 5.5), probably arising from phenolic compounds in the smoke deposits (Ferrer-Gallego et al., 2014). At the recorded perception levels, this aftereffect is less likely to be felt when the products are consumed in a meal.

Overall, the QDA findings suggest that product appearance, texture and mouthfeel are likely to be important determinants of consumer preference, since significant differences were found in those properties. The extent to which that was the case is discussed next.

5.2.2 Consumer acceptance: FTT product vs. Chorkor smoker product

Given the differences in the appearance, texture and mouthfeel of products from the FTT and Chorkor smoker (from the QDA results), the consumer acceptance test was conducted to determine which product was preferred.

5.2.2.1 Demographics of assessors

There were more males (59%) than females (41%) among the assessors. The majority (64%) of respondents were aged 18–24 years. Other demographic characteristics of the assessors are summarized in Appendix 3.

5.2.2.2 Mean sensory attribute scores

No significant differences were observed between the scores for the FTT and Chorkor smoker products for any of the sensory characteristics assessed (Fig. 5.6). This suggests that the QDA differences did not influence consumer response to the products. Thus, on the basis of the test results, consumers are likely to accept FTT products just as much as Chorkor smoker products.

It is significant to note that whereas assessors in QDA tests are trained to identify even subtle sensory differences in products (Stone et al., 2012), the everyday consumer engaged in consumer acceptance tests is not trained that way. Hence, as observed in this study, differences perceived in QDA tests may not always be picked up in consumer tests. For example, whereas the QDA showed that the softness attribute of texture differed significantly between products of the two ovens ($p=0.008$, the smallest p-value in that category), consumers did not identify any difference ($p=0.928$) in that attribute (Fig. 5.6).

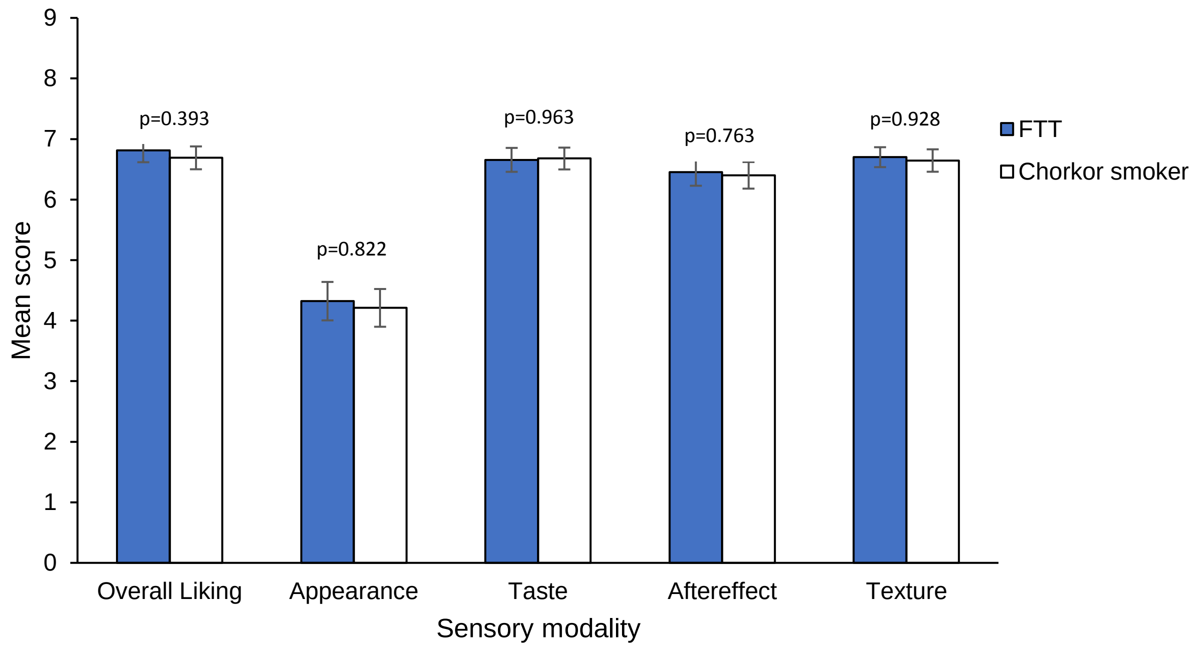

Fig. 5.6: Consumer liking scores (mean±SE) for the sensory modalities of smoked-soft mackerel (*Scomber japnicus*) produced on the FTT vs. the Chorkor smoker. p-values relate to comparisons between the ovens for each attribute

5.2.3 Product characteristics influencing consumer choice for smoked fish

Taste was reported as the most important sensory characteristic that drove consumer choice for smoked fish (68.1% of responses), followed by appearance (14.8%) and texture (12.5%) (Fig. 5.7). Taste, as used here, relates to the *a priori* knowledge of the typical (and thus expected) taste of a particular type of smoked fish, rather than a taste testing of the product to confirm that characteristic before purchase. This is true for the other sensory attributes.

Interestingly, price was listed as the least driver. This may be explained in part by the observation in the Ghanaian context that, on the whole, the type of smoked fish chosen depends largely on the type of meal to be prepared. The suitability of a given smoked fish product for a particular food depends mainly on its taste and flavour attributes with which consumers are already familiar. Therefore, at the point where a meal choice is made, the consumer is already prepared to pay the required price for the type of smoked fish suited to that meal.

Since the ultimate sensory appeal of a food product may be its taste, it is understandable that consumers considered this characteristic the most important choice driver. It is the (typical and expected) taste of the smoked fish that defines its suitability for particular food applications, and hence the decision of consumers to acquire the products for same. Price, then, becomes a secondary matter that may compel an alternative choice (also suited to the desired meal), should means be a challenge.

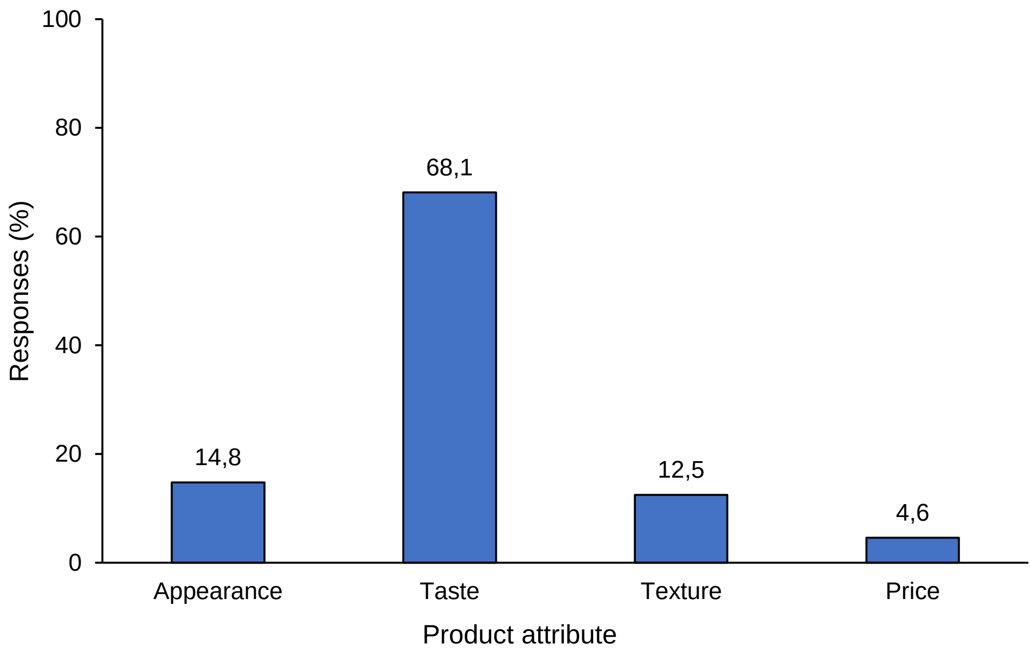

Fig. 5.7: Smoked fish attributes considered the most important influcencer of consumer choice

Considering that consumers found no difference (p=0.963) in the taste of the FTT and Chorkor smoker products (Fig. 5.3) and that this sensory property was the most important determinant of choice for smoked products (Fig. 5.7), it follows that consumers are likely to accept smoked fish from the FTT. Furthermore, it is probable that the acceptance will be increased if the food safety value addition of the products is properly communicated to the public (Mindjimba et al., 2019). It should be noted, however, that only one species of fish (mackerel) and one product type (smoked-soft) was evaluated, hence extrapolation of the results to all other smoked fish products may not necessarily be warranted.

5.3 CONCLUSION

Noticeable appearance, texture and mouthfeel differences are observed in smoked-soft mackerel separately processed on the FTT and Chorkor smoker. Whereas the Chorkor smoker product was darker in appearance, the FTT product was drier to the touch and harder on the palate. These differences notwithstanding, consumers did not show a significant liking of one product over the other, suggesting that the FTT product may be accepted at least to the same degree as the Chorkor smoker product.

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An exploratory study on the potential formation of epoxy- and other oxidised fatty acid species as indicators of lipid oxidation in smoked fish is discussed next in Chapter 6.

CHAPTER 6: EXPLORATORY CHARACTERIZATION OF EPA- AND DHA-DERIVED EPOXY FATTY ACIDS AS INDICATORS OF LIPID OXIDATION IN SMOKED FISH

ABSTRACT

This study determined the occurrence of methyl eicosapentaenoic acid (EPA)- and methyl docosahexaenoic acid (DHA)-derived epoxides in stored smoked fish as a function of smoking oven type and storage time, and evaluated the potential use of those epoxides as indicators of lipid oxidation in (smoked) fish lipids. *Sardinella* sp. was smoked-dry separately on the FTT, Chorkor smoker and metal drum oven in five replicates, stored and sampled at 0, 3 and 6 months for fat extraction by the Bligh and Dyer method and subsequent quantitative and qualitative fatty acid methyl ester (FAME) profiling on a gas chromatograph equipped with a flame ionization detector (GC-FID). The occurrence of EPA- and DHA-derived epoxy fatty acids in the extracted fat was determined by room-temperature base-catalysed transmethylation followed by solid phase extraction and injection of the polar fraction on GC-FID, and subsequently on GC-MS for identification based on mass spectral analysis of in-house epoxidized methyl EPA and methyl DHA reference standards. Six brands of dietary fish oil capsules were also placed in four treatments (as-is; three-week storage, thermoxidation and epoxidation) and profiled for FAME and the epoxides of interest. Polyunsaturated fatty acid (PUFA) content decreased over time in the FTT product ($p<0.05$) but remained relatively stable in the Chorkor smoker and metal oven products. A potential anti-oxidative effect of wood smoke phenolic compounds was cited. Four EPA- and five DHA-derived epoxide regioisomers were identified in all smoked fish samples. The epoxides were also detected in thermoxidized and epoxidized capsule samples but not in untreated and stored capsules. The findings suggest that whereas lipids in fish smoked on the Chorkor smoker and the metal drum oven are comparatively stable, those in FTT products undergo progressive oxidation during storage. The derived epoxides of methyl EPA/DHA occurred in surprisingly low amounts (within the limits of semi-quantitative estimation) and may thus not be considered suitable indicators of lipid oxidation in (smoked) fish oil.

Keywords: epoxy fatty acids; lipid oxidation; fish lipids; smoked fish

6.0 INTRODUCTION

As explained in Chapters 1 and 2, in Ghana, smoked-dry fish is stored for up to nine months without obvious physical deterioration due to their low moisture content and the practice of re-smoking. The storage is done by an unsophisticated method in which large quantities of the product are arranged on racks, covered with thick polyethylene sheets and kept under ambient tropical temperature conditions on the compound of the processors' home[4] (Fig. 6.1). In some instances, in lieu of the racks, the products are bulk-wrapped with brown paper and tied in baskets.

Fig. 6.1: Traditional storage of smoked-dry fish in the home of a processor. The products are arranged on racks, which are then stacked, covered with thick polyethylene sheets and tied. Some racks are visible at the base of the left stack.

The described storage condition and duration could pre-dispose lipids in the smoked fish to oxidative degradation. Fish lipids are rich in the polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are particularly susceptible to oxidation (Oterhals and Vogt, 2013; Cameron-Smith et al., 2015).

[4] Fish smoking in Ghana is typically a cottage business

As discussed in Chapter 1, although the peroxide value and levels of secondary oxidation products (such as aldehydes, ketones and oxo-fatty acids) are typically used as indicators of lipid oxidation (Schaich, 2005; Frankel, 2012;), new evidence suggests that the rarely considered modified, oxidized fatty acid species such as epoxides could be useful indicators. Mubiru et al. (2013) found unexpectedly high epoxy fatty acid amounts (up to 2mg per g of oil) in fresh vegetable oils that had low peroxide values (< 12.6 meq O_2 per g of oil). Thus, whereas the oils may have been considered minimally oxidized on the basis of their peroxide values, the high epoxy fatty acid contents suggested otherwise. This implies that the indicators traditionally used for oxidation in lipids may not always be reliable (Vandemoortele, 2020), and that the epoxides could be considered a sensitive indicator (Mubiru et al., 2013).

Given the predominance of PUFAs in fish oil, it was considered that such oils would be much more susceptible to the formation of the epoxy fatty acids than the vegetable oils. However, at the time of this study, no reports were found in the available scientific literature on the occurrence of epoxy fatty acids in fish as such nor in fish oil. Hence, in light of the findings of Mubiru et al. (2013) (i.e. high epoxy fatty acid content in apparently minimally oxidized vegetable oil), the lack of data on epoxides in fish oil (highly oxidation-prone commodity), and the susceptibility of lipids in stored smoked fish to oxidation, this work was conducted as an exploratory endeavour to attempt to determine the occurrence of EPA- and DHA-derived epoxy fatty acids in smoked fish lipids and evaluate their potential as indicators of lipid oxidation in that product.

Considering the potential food matrix effect on the extractability and purity of lipids from the smoked fish, dietary fish oil supplements were also evaluated to provide a picture of the occurrence of epoxy fatty acids in a simple but pure fish lipid matrix (i.e. since fish oils in supplement capsules do not require chemical extraction). It was envisaged that parallelly assessing the two fish lipid matrices would enable a better understanding of the situation under study, and thus explore the potential use of epoxy fatty acids as indicators of lipid oxidation in fish oil in general.

6.1 MATERIALS AND METHODS

The experimental procedures are summarized in Fig. 6.2. Batches of *Sardinella* sp. separately smoked-dry on the FTT, Chorkor smoker and the metal drum oven were stored for six months under traditional storage conditions. For each oven, products from five replicate smoking sessions were stored. Samples were taken at the beginning, in the third and sixth month of storage for fat extraction and subsequent profiling of the (oxidized) fatty acids in the extracted fat. Total smoked fish samples (hence fat extract samples) were 45 (3 ovens x 3 storage periods x 5 replicates = 45 samples).

Fish oil capsules (omega 3 fatty acid supplements) were also purchased from supermarkets in Ghent, Belgium, and profiled for (oxidized) fatty acids under four conditions: untreated, stored (for 3 weeks, decapsulated), thermoxidized and epoxidized. Total fish oil capsule samples were 24 (6 brands x 4 treatment groups = 24 samples).

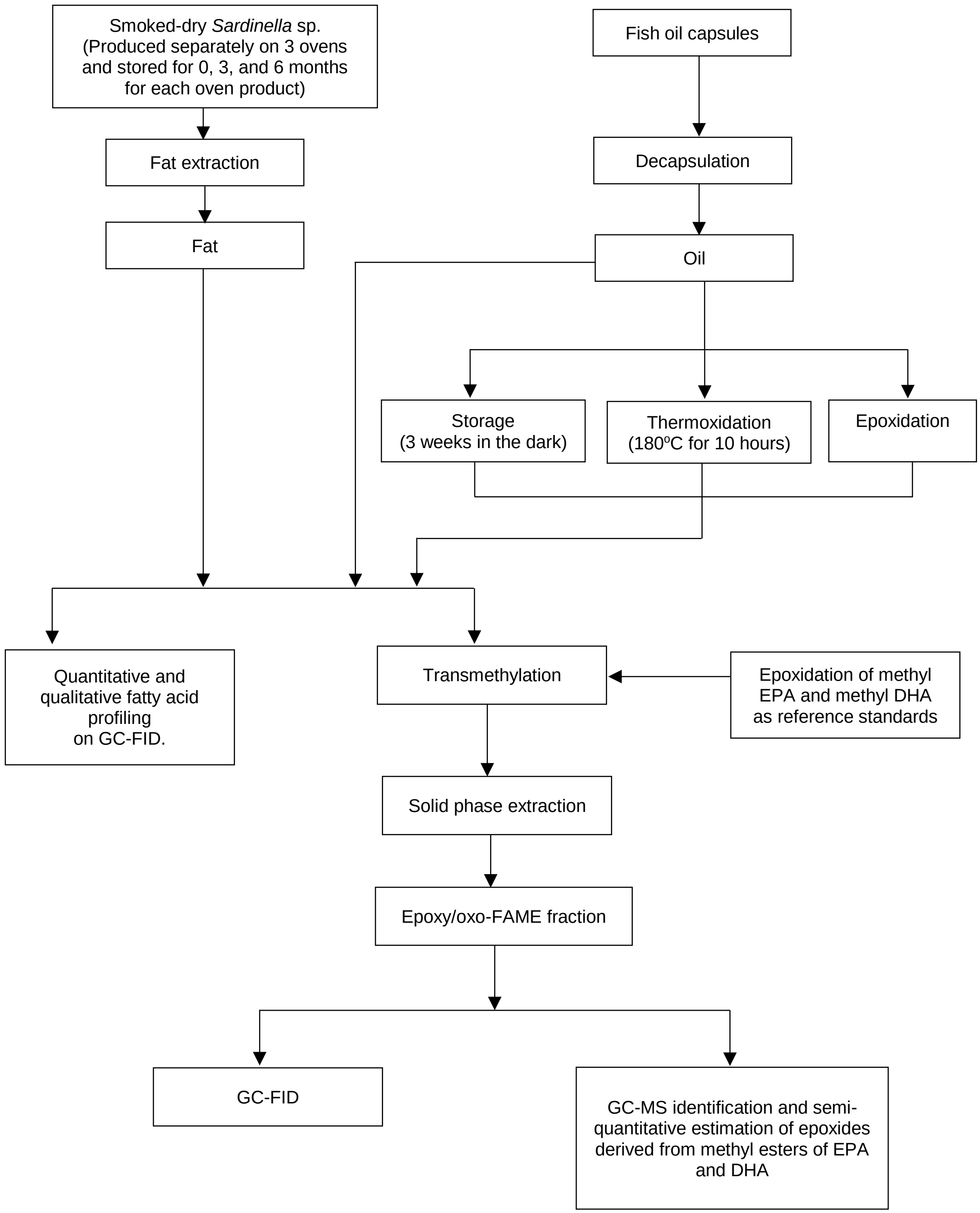


Fig. 6.2: Schematic overview of lipidomic study on extracted smoked fish fat and fish oil capsules

6.1.1 Smoked fish sample preparation

6.1.1.1 Sample preparation

At each sampling period (Month 0, Month 3 and Month 6), ten fishes were taken from the respective batches of products from the three ovens and homogenized as-is with a Waring® CB15V Heavy Duty Commercial blender (Waring Commercial, Stamford, CT, USA). The dry homogenates were vacuum-packed with Henkelman® JUMBO 42 vacuum packaging machine (Henkelman USA, Elmhurst, IL, USA) and kept frozen at -22ºC until analyzed.

6.1.1.2 Moisture content determination

Moisture contents were determined by the oven-drying method (AOAC, 1999). Approximately 5 g of the homogenized sample was weighed and stirred into glowed sea-sand (pre-dried and cooled) in an aluminium dish (pre-dried and cooled) and placed in an air oven at 105ºC for two hours. The dishes were cooled in a desiccator and the weight difference noted. The drying was repeated at 30 minutes intervals until a constant weight was obtained. The moisture content was expressed in weight percentage (g moisture/100 g product).

6.1.1.3 Fat extraction

The Bligh and Dyer (1959) method of lipid extraction was applied, with slight modifications. The method was specifically designed for a rapid and efficient extraction of lipids under mild conditions to minimize oxidative decomposition (Bligh and Dyer, 1959).

For each sample, fat was extracted from a total of 10 g of product. Due to practical limitations of centrifuge tube sizes (smaller capacity than required total solvent volumes), four extractions were done on 2.5 g portions of each product and the extracts were subsequently combined. Each 2.5 g of homogenized fish was weighed directly into a centrifuge tube and the extraction solvents added in the following order and ratio: water (volume depending on moisture content), dichloromethane (11 mL) and methanol (26 mL). The water added was calculated based on a requirement of 14.5 g water per 2.5 g of a sample having 0% moisture (Mubiru et al., 2014). (This adjustment served to augment final phase separation (Mubiru et al., 2014)). The sample-solvent mixture was homogenized with an Ultra-Turax (Janke & Kunkel, IKA-Werk, Staufeb, Germany) for 2 min, followed by addition of 15 mL dichloromethane and homogenizing for 30 seconds. A 15 mL amount of acidic water (pH ≤2, acidified with 6 M hydrochloric acid) was added, the content homogenized for 30 seconds and centrifuged at 2975 G-force at 4^{o}C for 10 min on a Rotina 380R centrifuge (Hettich, Eeklo, Belgium). The pH of the aqueous (upper) layer was checked to ensure it was lower than 2. If higher, it was lowered by addition of 5M HCl and the homogenization and centrifugation repeated. The aqueous layer was decanted with a glass pipette and the organic layer filtered through anhydrous sodium sulphate into a pre-dried and cooled flat-bottom flask. For each sample, the organic layer of the extractions on the four 2.5 g portions were filtered into the same flask. The solvent was evaporated on a rotavapor (Heidolph, Schwabach, Germany) at ≤ 30^{o}C. The flask was dried further under nitrogen, weighed, and the percentage fat content calculated relative to the original combined weight of the four portions as:

$$\%Extracted\ fat = \left(\frac{Weight\ of\ flask\ with\ extract - Weight\ of\ empty\ flask}{Sample\ weight}\right) x100 \quad (1)$$

6.1.2 Procurement and preparation of fish oil capsules

Six brands of dietary fish oil capsule supplements were purchased from supermarkets in Ghent, Belgium. The brands (as labelled on the products) were Biover Omega 3 (Biover, Nazareth, Belgium), Boni Omega 3 (Boni selection, Halle, Belgium), Lucovitaal Omega 3 (Lucovitaal, Eindhoven, Netherlands), Vitarmonyl (Laboratoires Vitarmonyl, Tournai, Belgium), Kruidvat Omega 3 Capsules (Kruidvat, Antwerp, Belgium) and Krill Omega 3 (Orca natural solutions, Zedelgem, Belgium).

The oil in the capsules were extracted with a hypodermic syringe into glass vials and a portion analysed as-is (untreated) or subjected to three different treatments: storage for three weeks, thermoxidation or epoxidation. For the storage treatment, the decapsulated oils were stored in tightly closed glass vials (2.5 mL vials, filled to the mark) in the dark for three weeks at room temperature. For the thermoxidation, the decapsulated oils were heated in a muffle furnace at 180ºC for 10 h. The epoxidation treatment was done based on the procedure by Gunstone and Jacobsberg (1972) as described below.

6.1.2.1 Epoxidation of decapsulated fish oil

Approximately 200 mg of oil was weighed into a pear-shaped flash pre-covered with aluminium foil (to provide dark conditions) and reacted with 160 mg of 3-chloroperbenzoic acid in 20 ml of dichloromethane for 4 hours at room temperature. The dichloromethane was evaporated with a rotavapor and n-hexane added to the residue (to dissolve the oil) and filtered over anhydrous sodium sulphate into amber vials. The vials were dried under nitrogen, closed and kept at -20ºC for further analysis.

6.1.3 Determination of fatty acid profile on GC-FID

The fatty acid composition of the samples was determined by gas-liquid chromatography according to the AOCS (1990) method Ce 1b-89 for marine oils. The triacylglycerols were saponified in a capped test tube with methanolic sodium hydroxide solution followed by esterification of the fatty acids with boron trifluoride in methanol (BF_3/MeOH). The resulting fatty acid methyl esters (FAMEs) were identified and quantified by gas chromatography. For the epoxidized capsule oils, FAME was determined for Biover. Single FAME measurements were done for all capsule samples.

6.1.3.1 Internal and reference standards for FAMEs

Two standards were used for the FAMEs; one for the identification and the other for quantification. Identification was done using a reference standard of a mixture of FAMEs (GLC 68D, Nu-Chek Prep, Inc., USA). Nonadecanoic acid (C19:0) was used as an internal standard (IS) for the quantification. The IS (0.5 mL from a 5 mg/mL stock in isooctane) was placed in test tubes and dried under nitrogen before fat samples were weighed into the same tubes for the saponification and esterification.

6.1.3.2 Saponification and esterification of samples

Approximately 25 mg of fat sample (extracted smoked fish fat or untreated/treated decapsulated fish oil) was weighed into a screw cap test tube containing the dried IS.

Methanolic NaOH (0.5M) solution was added (2 mL) and the test tube tightly closed and placed in a boiling water bath for 7 mins for the saponification reaction. The test tube was cooled under running water. 2 mL of BF_3/MeOH was added, the reaction system mixed for 30 seconds on a vortex mixer (VWR International, Leuven, Belgium) and subsequently placed in the boiling water for 5 mins for the esterification.

The tubes were cooled after the esterification and 3 mL of isooctane was added, followed by vortex mixing for 30 seconds and addition of 5 ml of saturated aqueous NaCl. The tube was vortexed for another 30 seconds and allowed to stand for phase separation. The upper isooctane layer (containing the FAMEs) was transferred to another test tube containing a layer of anhydrous sodium sulphate at the bottom. The extraction was repeated with 3 mL of isooctane. The final extract was vortexed for 15 seconds and dissolved in isooctane (160 µL extract in 840 µL of solvent) for GC analysis. The GLC standard was loaded (in a separate vial) along with the samples.

6.1.3.3 Gas chromatograph FID conditions

The FAMEs were analysed with an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID)(G1530N) (Agilent Technologies, Santa Clara, USA) by a cold on cold (COC) injection (0.1µL sample) on a CP-Sil 88^{TM} column for FAME (Agilent Technologies, Santa Clara, USA). The column measured 60 m by 0.25 mm and had a capillary coated with 0.2 µm film. A deactivated fused silica pre-column (3 m x 0.25 mm) (Agilent, Belgium) was fitted to protect the column. The oven temperature programme used was: 50ºC hold for 4 min, then ramp to 225ºC at 12ºC min^{-1} and hold for 25 min. The FID temperature was set at 300ºC. Gas flow rates for hydrogen, air and helium (makeup gas) were 40, 400 and 20 mL min^{-1}, respectively.

6.1.3.4 Identification and quantification of FAMEs

Using a Microsoft Excel-based peak integration template, the FAMEs in the samples were identified by a comparison of their retention times with those of the known FAMEs in the GLC standard. Where no corresponding peaks were found in the GLC standard, efforts were made to identify the unknown peak by gas chromatography mass spectrometry (GC-MS, described later in Section 6.1.4.3.2).

The FAMEs were quantified using the aforementioned integration template, based on the amount of IS added, the sample weight and the peak areas. FAME content was reported as g fatty acid/100 g of extracted fat for smoked fish samples, and as g fatty acid/100 g of fat for capsule samples.

6.1.4 Determination of epoxy fatty acids in extracted smoked fish fat and fish oil capsules

The occurrence of EPA- and DHA-derived epoxy fatty acids in the samples was determined according to the protocol described by Mubiru et al. (2013). The method involves room temperature base-catalysed transmethylation of the fatty acids followed by a 3-step solid phase extraction (SPE) with three n-hexane:diethyl ether solvent systems of increasing polarity.

6.1.4.1 Base-catalysed transmethylation

An amount of 200 mg of fat sample was weighed into a 25 mL glass centrifuge tube. 5 mL of tert-butyl methyl ether (tBME) and 0.2 M sodium methoxide solution in methanol were added, the mixture vortexed for 1 min and allowed to stand at room temperature for 2 min. Then, 0.17 mL of 0.5 M sulfuric acid was added, and the mixture vortexed for 15 seconds. Finally, 5 mL of distilled water was added and the tube vortexed for 30 seconds and centrifuged at 3600g for 1 min. The organic layer was filtered through anhydrous sodium sulphate into a pear-shaped flask. The extraction was repeated two more times with 5 mL of tBME at each instance. The filter was rinsed with 15 mL of tBME into the pear-shaped flask. The solvent was evaporated on a rotavapor and the FAMEs dried under nitrogen.

6.1.4.2 Solid phase extraction (SPE)

Silica gel was dried in a muffle furnace at 450ºC for 12h and cooled in a desiccator. The moisture content was adjusted to 10% and equilibrated for 1hr on a shaker. This activated silica gel was sealed, kept in a desiccator and used within two weeks.

6.1.4.2.1 Preparation of the silica column

The column was prepared by weighing 1 g of the activated silica gel into an SPE cartridge (6mL, 6.5cm×1.3cm). 3 mL of n-hexane:diethyl ether (98:2, v/v) was added and the resulting slurry stirred well to prevent formation of air bubbles. The column was also tapped to remove any air bubble. The gel was allowed to settle, and the column filled to the brim with the elution solvent. About 0.5 cm of glowed and cooled sea-sand was gently added to cover and protect the column surface. The solvent was then drained until just enough amount remained to cover the sand surface.

6.1.4.2.2 Extraction of polar FAME fraction

The n-hexane:diethyl ether solvent systems developed by Mubiru et al (2013) for optimized SPE are 98:2 (v/v), 90:10 (v/v) and 70:30 (v/v). These solvents elute the non-polar fraction (comprising unaltered fatty acids), the epoxy/oxo-fatty FAMEs, and the hydroxyl FAMEs, respectively, in that order. Since the interest of this study was in the epoxy/oxo-FAME fraction, the first two solvent systems were used.

The dried FAMEs in the pear-shaped flask were dissolved in 2 mL of the 98:2 (v/v) solvent system and loaded unto the silica column. The non-polar fraction was eluted with 15 mL of this solvent, gently adding 1 mL of solvent at a time and carefully washing the inner sides of the column with the same solvent to ensure optimal elution of the non-polar fraction. After this, 15 mL of the 90:10 solvent system (15 mL) was used to elute the polar epoxy/oxo-FAME fraction into a separate test tube. The eluted fraction was transferred into pre-weighed amber vials, dried under nitrogen and the net weight noted. The dried FAMEs were stored at -22ºC for GC analysis.

6.1.4.3 Identification of epoxy FAMEs

For the identification of the EPA- and DHA-derived epoxides, an in-house reference standard was prepared by epoxidizing methyl EPA and methyl DHA (Nu-Chek-Prep., Inc. USA) following the Gunstone and Jacobsberg (1972) method described earlier. The reaction time of 4 hours was strictly observed to limit epoxide formation to monoepoxides. It was expected that only epoxides of the respective FAMES would be formed. Thus, the potential presence of epoxides in the fat samples (smoked fish and capsules) could be determined by comparing the retention times of the polar fractions of the samples with those of the reference standard on the GC-FID.

6.1.4.3.1 Injection of epoxy/oxo-FAMEs on GC-FID

The polar FAMEs of the reference standard (and later the samples) were injected on the GC-FID to scope for the presence of EPA- and DHA-derived monoepoxides (hereafter called epoxides) and to optimize a temperature programme for their elution. The same GC-FID equipment and gas flow rates described in Section 2.3.3 were used. The temperature programme was, however, adjusted as follows: hold at 50°C for 1 min, then ramp to 225°C at 40°C/min and hold for 45 min. This temperature programme optimisation was done to reduce the retention time (facilitate earlier elution) of the epoxy compounds.

6.1.4.3.2 Injection and identification of epoxy FAMEs on GC-MS

The identity of suspected epoxy peaks in the reference standard were evaluated by gas chromatography-mass spectrometry (GC-MS). The FAMEs were injected in an Agilent 7890A GC equipped with a 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA). The same column and chromatographic conditions used on the GC-FID for the epoxy/oxo-FAMEs were applied. A split/spitless injector in spitless mode was used and the injection volume was 1 µL. The MSD conditions were: capillary direct interface temperature 250°C, ionization energy 70eV, operating scan mode between *m/z* 30 and *m/z* 600 at a scan rate of 3.64 cycles/second. The mass spectra of the peaks were examined for the presence of characteristic ions (*m/z* values) expected from the fragmentation of EPA and DHA epoxides.

Three fragmentation patterns were considered, based on the work of Orellana-Coca et al. (2005) and Christie (2018), as illustrated in Fig. 6.3. When an epoxide was confirmed to be present, the relative abundances of the characteristic ions and other ions present at ≥30% were reported.

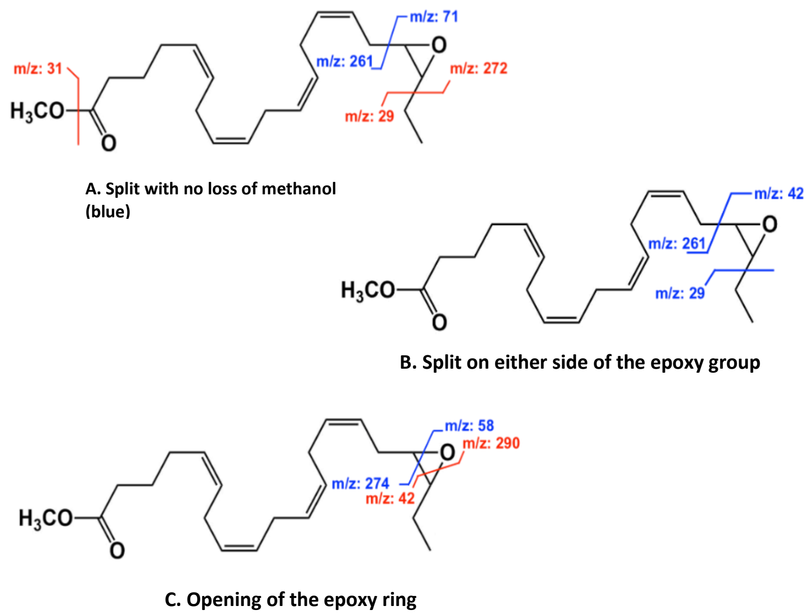


Fig. 6.3: Illustration of fragmentation patterns for methyl 17,18-epoxy-5,8,11,14-eicosatetraenoate

The fragmentation patterns in Fig. 6.3 were made for each of the regioisomers of EPA and DHA monoepoxides. The resulting *m/z* values are summarized in Table 6.1. The mass spectra of the suspected epoxy peaks were scrutinized for the presence of characteristic *m/z* values among the fragmentation ions.

Table 6.1: Ions (m/z) expected from the fragmentation of monoepoxides derived from methyl EPA and methyl DHA (according to the fragmentation patterns described in Fig. 6.3)

Group of epoxides	Regioisomer	Fragmentation ions (*m/z*)
Methyl EPA monoepoxides	Methyl 5,6-epoxy-8,11,14,17- eicosatetraenoate	**112**, **130, 218, 231, 189**, *31, 41, 101, 114, 202*,
	Methyl 8,9-epoxy-5,11,14,17- eicosatetraenoate	**152, 170, 178, 191,** *31, 42, 141, 149, 154, 162*
	Methyl 11,12-epoxy-5,8,14,17- eicosatetraenoate	**138, 151, 192, 210,** *31, 42, 109, 122, 181, 194*
	Methyl 14,15-epoxy-5,8,11,17- eicosatetraenoate	**98, 111, 232, 250,** *31, 42, 69, 82, 221, 234*
	Methyl 17,18-epoxy-5,8,11,14- eicosatetraenoate	**58, 71, 272, 290,** *29, 31, 42, 261, 274*
Methyl DHA monoepoxides	Methyl 4,5-epoxy-7,10,13,16,19-docosapentaenoate	**98, 116, 258, 271,** *31, 42, 87, 100, 229, 242*
	Methyl 7,8-epoxy-4,10,13,16,19- docosapentaenoate	**138, 156, 218, 231,** *31, 42, 127, 140, 189, 202*
	Methyl 10,11-epoxy-4,7,13,16,19- docosapentaenoate	**178, 191, 196,** 31, *42, 149, 162, 167, 180*
	Methyl 13,14-epoxy-4, 7,10,16,19- docosapentaenoate	**138, 151, 218, 236,** *31, 42, 109,122, 207, 220*
	Methyl 16,17-epoxy-4, 7,10,13,19- docosapentaenoate	**98, 111, 258, 276,** *31, 42, 69, 82, 247, 260*
	Methyl 19,20-epoxy-4, 7,10,13,19- docosapentaenoate	**58, 71, 298, 316,** *29, 31, 42, 287, 300*

Bold = characteristic ions, Italics = other fragmentation ions

Although regioisomers can be distinguished on the basis of their mass spectra, geometric (*cis-trans*) isomers cannot. However, on the CP Sil 88^{TM} column used in this study, *trans* isomers elute first (Mubiru et al., 2013), thus allowing a differentiation to be made.

6.1.4.4 Semi-quantitative estimation of epoxy occurrence

Since no internal standard was used for the epoxy FAMEs (due to the exploratory nature of the work), a semi-quantitative approach was adopted to estimate the occurrence of epoxides using the peak areas. Four relative peak areas were calculated. For example, for methyl EPA-derived epoxides, the peak area of each regioisomer was expressed as a percentage of the total area of the peaks for that group of compounds. The same was done for the methyl DHA-derived epoxides. Secondly, the total peak area of all methyl EPA-derived epoxides was expressed as a percentage of the total area of all peaks in the polar fraction. This was repeated for the methyl DHA-derived epoxides.

6.1.5 Statistical analysis

The quantitative data of extracted fat and their corresponding FAME contents were analysed using SPSS Version 21 (IBM Corp., Armonk, NY, USA). The data were first explored for normality by the Shapiro-Wilk test and found not to show significant departure from a normal distribution. Therefore, ANOVA was used to compare the means. Since two factors (oven type and storage time) were considered at three levels each (FTT, Chorkor smoker and metal drum oven for “oven type factor” and 0, 3 and 6 months for “storage time factor”), a 2-way ANOVA was applied to compare the means. Statistical significance was set at 0.05.

6.2 RESULTS AND DISCUSSION

6.2.1 Overview

The extracted fat contents and fatty acid profile of the smoked fish products are given in Table 6.2. Fat contents are presented as g/100 g of dry matter, and FAMEs as g fatty acid/100g of extracted fat. The FAME are also reported on g/100 g dry matter of smoked fish and briefly discussed. Across the oven types, the mean extracted fat amounted from 7.4 to 14.2 g/100 g dry matter (Table 6.2), with the metal drum recording higher figures ($p<0.05$) than the other ovens. No significant differences ($p>0.05$) were found in the fat content of FTT products (for all storage months) and Chorkor smoker products (for all storage months). However, for the metal drum oven products, the fat content in Month 0 was significantly higher than in Month 3 ($p=0.00001$) and in Month 6 ($p= 7.0 \times 10^{-7}$). Fat extracts from products are hereafter named by the oven type (e.g. “FTT extract” denotes fat extracted from the FTT product).

For the fatty acid profiles (Table 6.2), generally, PUFA levels in FTT extracts showed significant reductions ($p<0.05$) over the storage period than the Chorkor smoker and metal drum extracts. MUFA levels were generally more stable, with no significant decreases ($p>0.05$) over the storage period for specific ovens. However, for month-on-month comparisons among the ovens, MUFAs in the FTT extract at Month 6 were lower than in the Chorkor smoker ($p=0.0004$) and the metal drum oven ($p=5.0\times10^{-12}$) product at the same month. Similar observations were made in Month 3. This suggests a degradation of the MUFAs in the FTT products over time.

To ascertain the contribution of oven type and storage period on the differences in unsaturated fatty acid levels, the statistical main effects and interaction effects were evaluated. For MUFAs, main effects were found for both oven type (accounting for 88.7% of variances at $p=2.0\times10^{-16}$) and storage period (17.3%, $p=0.044$). No interaction effect ($p=0.189$) was observed. Similar to the MUFAs, oven type had a significant main effect

(52%, p=6.0x10^{-6}) for PUFAs. However, there was no significant main effect (p=0.281) for storage period, whiles the interaction effect was significant (64.7% at p=4.0x10^{-7}).

It is seen, then, that for both MUFAs and PUFAs, oven type had a significant impact on the degree of unsaturated fatty acid content reduction (indicative of oxidative degradation), and that this occurred to the highest extent in the FTT extracts. The observed interaction effect for the PUFAs suggests that, under the traditional storage conditions and for the same duration, PUFAs in FTT products are likely to be more oxidized than the Chorkor smoker and the metal drum oven products.

Derived-epoxides of EPA and DHA were identified in all smoked fish fat extracts. For the capsule oils, those epoxides were found in some thermoxidized samples and all epoxidized samples, but not in the untreated and stored samples (Table 6.5).

In subsequent sections, each assessed parameter is considered in detail. For oxidative stability discussions, PUFAs are highlighted, due to their relevance as the key unsaturated fatty acids in fish lipids (Kris-Etherton et al., 2002; Secci et al., 2016), and given that the in-house reference standard for epoxy identification was synthesised from PUFA methyl esters. The summary tables of all the 2-way ANOVA analyses are presented in Appendix 5.

6.2.2 Extractability of fats in smoked fish from the three ovens

In Table 6.2, it is noted that the metal drum fat extracts were higher than the FTT (p=7.8x10^{-11}) and Chorkor smoker (p=1.2x10^{-9}) for Month 0. Similar observations were made for Month 3 (p=8.4x10^{-7} for comparison with the FTT, and p=3.2x10^{-5} for comparison with the Chorkor smoker). This may be explained by considering potential differences in heat exposure during processing. Although this was not measured during the smoking experiments, it is probable that on the metal drum oven, the heat exposure per kg of fish is higher than is the case in the FTT and the Chorkor smoker, since only one (maximum three) layer of fish is smoked at a time, with poor control over heat distribution. Indeed, high incidences of product charring and non-uniform cooking were contributory motivating factors for the introduction of first-generation improved ovens to replace the metal drum oven (Nerquaye-Tetteh, 1999; Chapter 1). On that basis, it could be considered that the high heat exposure may have caused the bound lipids (membrane- and protein-bound) to be more easily extractable due to membrane disruption and protein denaturation. This, however, needs empirical verification by direct measurement of heat impact associated with each oven.

6.2.3 Fatty acid profile of extracted fat from the smoked fish

The total fatty acid content of the extracted fat (across ovens and storage periods) ranged from 55.3 to 75.6 g/100g. In the oils from the capsule, amounts up to 95.4 g/100 g were found. The lower amount in the smoked fish samples could be explained by a number of phenomena. First, in a pure tri-acylglycerol such as tri-stearin, the fatty acid content on 100 g lipid basis amounts to 95%. If there is a higher content of mid- and short chain fatty acids present, the fatty acid content drops and, therefore, typically, an amount between 90 to 95 g of fatty acids per 100 g of triacylglycerols is generally expected. If other substances (e.g. unsaponifiable matter) are present, the fatty acid content per 100 g of lipids would drop below 90 g/100 g. *Sardinella* sp. lipids have been found to contain up to 19% unsaponifiable matter (chiefly sterols) (Njinkoue et al. 2002), thus limiting the actual amount of fatty acids to be found in the extracted fat.

Secondly, the presence of (a large amount of) phospholipids may also reduce the fatty acid content. Njinkoue et al. (2002) reported phospholipid (principally phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol) contents of 6% in the extracted fat of *Sardinella* sp. In a phospholipid, only two fatty acids per molecule are present, thereby reducing the fatty acid content by about one third. It can be speculated that in all the extracts studied, part of the lipids consisted of phospholipids. It seems unlikely, however, that the differences in the fatty acid content are solely due to the presence phospholipids. Indeed, it was speculated before that membrane lipids (which are typically phospholipids) would be extracted more efficiently in the metal drum smoked fishes because of the presumed higher heat impact. Nevertheless, these extracts contained a higher amount of fatty acids per 100 g of extract. For example, from Table

6.2, it is noted that the total fatty acids in metal drum extract was more than in the FTT ($p= 8.8x10^{-7}$ for month 3) and the Chorkor smoker extract (p=0.0002 for month 3).

A third explanation for a drop in the fatty acid content in a lipid extract is the presence of oxidized and polymerised lipid species. It has been observed, for instance, that abused frying oils, with a polar content near or above 25%, contained a fatty acid content lower than 75% (*unpublished results*). This would suggest that the FTT extract would contain a considerable amount of oxidized species.

Considering the other results obtained, it remains currently unclear which of the above-mentioned phenomena was dominating and finally determining the actual total fatty acid content of the extracts. The data show, however, that in general, care should be taken in analysing lipid extracts from complex matrices such as smoked fish. It is only possible to analyse what is actually extracted. The extractability, however, may be affected by lipid interactions with the matrix. This was the basis for the parallel analysis of the decapsulated fish oils with respect to the occurrence of epoxy fatty acids, as that allowed avoidance of extractability challenges.

In the FTT extract, a significant reduction was observed in PUFA levels from Month 0 to Month 3 (p=0.012) and from Month 3 to Month 6 (p=0.001). The difference between Months 0 and 6 was even more significant ($p=7.0x10^{-7}$). The magnitudes of these period-to-period differences (reflected in the reducing p-values over increasing storage time) is an indication of progressive oxidative degradation of PUFAs during the traditional storage.

The observed lower extent of PUFA degradation in the Chorkor smoker and metal drum extracts (and by extension in the products themselves) could partly be explained by the more intense smoke exposure given to the products during processing (Chapters 2 and 3). Wood smoke is rich in phenolic compounds, which are known to have anti-oxidative effects on food lipids (Zamora and Hidalgo, 2016), for example through radical scavenging. In Cameroun, Marc et al. (1997) compared the impact of traditional fish smoking and cold smoking on lipid stability in smoked fish and reported a significantly lower rate of oxidation in the traditional product, corresponding with higher phenolic compounds (up to 14 times) in those products. Thus, while the FTT reduces PAH contamination by limiting the smoke deposition on products (among other factors, Chapter 3), this feature could enhance lipid oxidation *if* the products of the oven are stored under the described traditional conditions.

Across the oven types, it could be expected that the impact of the heat treatment on the haemoglobin in the fish could accelerate lipid oxidation. Haemoglobin is known to be an important pro-oxidant in fish muscle, acting through the release of activated oxygen and iron (Maqsood et al., 2012). Iron is among trace metals recognized for their significant pro-oxidative character (Schaich, 2005; Frankel et al., 2012). Thus, the released iron (and activated oxygen) could promote the oxidation of the fish lipids in the smoked fish. This effect should be larger in products from ovens with more intense heat treatments. However, it is noted that the metal drum oven extract (expected to suffer the highest heat impact) did not record that corresponding drop in PUFA levels. It is probable that the anti-oxidative effect of phenolic compounds in the smoke provided shielding for the lipids than was the case in the FTT extract (less smoke, less phenols).

A simplified summary of the changes in mean PUFA levels determined in fat extracts of the stored smoked fish is provided in Table 6.3, highlighting the magnitude of the statistical differences.

The fatty acid profile per dry weight of smoked fish is also presented in Table 6.4. and a summary of the related statistical differences in Table 6.5. In general, as previously observed for the extract basis, PUFAs in FTT products were less stable. However, it is noted that significant decreases in PUFA occurred in products of the Chorkor smoker ($p = 0.001$) and metal drum oven (0.004) as well, in month 3. These suggest that, as far as PUFAs are concerned, the nutritional quality of the hot-smoked fish decreases during traditional storage.

Table 6.2: Fatty acid profile of extracted fat of stored smoked-dry *Sardinella* sp. produced on the different ovens (presented on g/100g of extracted fat basis)

Parameter			FTT			Chorkor smoker			Metal drum oven		
			Month 0	Month 3	Month 6	Month 0	Month 3	Month 6	Month 0	Month 3	Month 6
Moisture content (g/100g of smoked fish)			16.4^{3,6,C,M}	11.9^{0,6}	13.3^{0,3,C,M}	11.6^{6,F,M}	11.7^{6}	12.5^{0,3,F}	13.0^{3,F,C}	11.8^{0,6}	12.1^{3,F}
Extracted fat (g/100 dry matter of smoked fish)			7.9^{M}	6.5^{M}	6.9^{M}	8.6^{M}	7.4^{M}	8.4^{M}	14.2^{3,6,F,C}	10.7^{0,F,C}	10.0^{0,F,C}
Mean SFA (g/100g of extracted fat)	C10:0	Capric acid	0.7	0.8	0.6	0.5	0.6	0.6	0.6	0.5	0.6
	C12:0	Lauric acid	1.1	0.9	0.8	0.9	0.8	0.8	0.8	0.8	1.0
	C14:0	Myristic acid	5.4	5.0	5.5	6.2	6.2	6.7	7.7	8.0	8.6
	C16:0	Palmitic acid	15.5	14.9	14.6	15.2	15.5	15.7	15.4	16.4	17.7
	C18:0	Stearic acid	4.3	4.1	4.0	3.9	4.1	4.0	3.6	4.3	4.7
	C20:0	Arachidic acid	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.6
	C22:0	Behenic acid	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
	C24:0	Lignoceric acid	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3
	Total SFA		**27.9**	**26.6^{M}**	**26.2^{C,M}**	**27.5^{M}**	**28.1^{M}**	**28.7^{F,M}**	**29.2^{3,6,C}**	**31.0^{0,6F,M}**	**33.5^{0,3,F,C}**
Mean MUFA (g/100g extracted fat)	C14:1	Myristoleic acid	0.8	0.7	0.7	0.7	0.8	0.7	0.6	0.6	0.7
	C16:1	Palmitoleic acid	4.4	3.9	4.1	5.1	5.1	5.6	7.1	6.8	7.3
	C18:1c9	Oleic acid	3.0	3.0	3.1	3.0	3.1	3.3	3.6	3.0	3.3
	C18:1c11	*cis*-Vaccenic acid	2.6	2.2	2.0	2.2	2.1	2.2	2.9	3.5	3.7
	C20:1	Gondoic acid	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2
	C22:1	Erucic acid	0.04	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	C24:1	Nervonic acid	0.3	0.3	0.4	0.4	0.4	0.4	0.2	0.3	0.3
	Total MUFA		**11.3**^{M}	**10.4^{C,M}**	**10.6^{C,M}**	**11.7^{M}**	**11.7^{F,M}**	**12.5**^{F,M}	**14.8^{F,C}**	**14.5^{6,F,C}**	**15.6^{F,C}**
Mean PUFA (g/100g of extracted fat)	C18:2 ω6	Linoleic acid	1.1	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.1
	C18:3 ω3	Linolenic acid	0.7	0.6	0.5	0.6	0.6	0.6	0.4	0.5	0.5
	C20:2 ω6	Eicosadienoic acid	0.8	0.6	0.6	0.8	0.7	0.7	0.8	0.9	0.9
	C20:3 ω6	Dihomogammalinolenic acid	0.1	0.1	0.1	n.d.	0.1	nd	0.1	0.1	0.1
	C20:4 ω6	Arachidonic acid	1.5	1.3	1.1	1.6	1.5	1.6	1.9	2.1	2.2
	C20:4 ω3	Eicosatetraenoic acid	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2
	C20:5 ω3	Eicosapentaenoic acid	4.3	3.5	3.1	4.8	4.3	47.	6.6	6.8	7.2
	C22:4 ω6	Docosatetraenoic acid	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
	C22:5 ω6	Docosapentaenoic acid ω6	0.7	0.6	0.5	0.6	0.6	0.6	0.4	0.5	0.6
	C22:5 ω3	Docosapentaenoic ω3	0.5	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.7
	C22:6 ω3	Docosahexaenoic acid	9.1	8.4	6.9	8.9	8.2	8.0	5.3	6.9	7.4
	Total PUFA		**18.9**^{3,6}	**16.8**^{0,6,M}	**14.1**^{0,3,C,M}	**18.9**^{M}	**17.6**^{M}	**17.9**^{F,M}	**17.3^{3,6,C}**	**19.6**$^{0.F,C}$	**21.0**^{0,F,C}
Unidentified fatty acids (g/100g extracted fat)			5.5	4.4	4.3	4.9	4.4	4.3	5.1	5.2	5.5
Total fatty acids (g/100g of extracted fat)			**63.5^{3,6}**	**58.1^{0,M}**	**55.3^{0,C,M}**	**62.3**	**61.9^{M}**	**63.4^{F,M}**	**66.4^{6}**	**70.2^{F,C}**	**75.6^{0,F,C}**

SFA = saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; nd=not detected

For each fatty acid, values are means of 5 replicates. Relative standard deviations (not shown) of individual measurements were typically less than 20%.

Where used, superscripts indicate significant difference at $p<0.05$. Numerical superscripts represent a comparison among months for the same oven, and the letter superscripts for comparison among the three ovens for the same month. For the numerical superscripts, 0=different from month 0; 3=different from month 3; 6 = different from month 6. For the letter superscripts, F= different from FTT; C=different from Chorkor smoker; M = different from metal drum oven.

Table 6.3: Summary of differences in changes in PUFA levels in smoked-dried *Sardinella* sp. produced on different ovens and stored for up to 6 months (assessed per extracted fat from smoked fish)

Factor	Level	Comparison	p-value	Difference in mean PUFA level	Comment on stability of PUFAs
Same oven, different months	FTT	Month 0 vs Month 3	0.012	Less in month 3	Oxidative degradation with time
		Month 0 vs Month 6	0.000001	Less in month 6	Oxidative degradation with time
		Month 3 vs Month 6	0.001	Less in month 6	Oxidative degradation with time
	Chorkor Smoker	Month 0 vs Month 3	0.096	None	PUFAs stable
		Month 0 vs Month 6	0.210	None	PUFAs stable
		Month 3 vs Month 6	0.737	None	PUFAs stable
	Metal Drum	Month 0 vs Month 3	0.005	None	PUFAs stable
		Month 0 vs Month 6	0.0001	No reduction. More in month 6*	PUFAs stable
		Month 3 vs Month 6	0.083	No reduction. More in month 6*	PUFAs stable, but extractable fat drops (Table 6.2), indicating overall lipid oxidation and/or potential reaction with proteins
Same month, different ovens	Month 0	FTT vs Chorkor	0.992	None	-
		FTT vs. Metal drum	0.051	None	-
		Chorkor vs. Metal Drum	0.040	Less in Metal drum	More oxidation in metal drum product, potentially due to higher heat exposure. Statistical significance not strong
	Month 3	FTT vs Chorkor	0.276	Not significant	
		FTT vs. Metal drum	0.0009	Less in FTT	Oxidative degradation in FTT product
		Chorkor vs. Metal Drum	0.015	Less in Chorkor smoker	Higher extractable fat and PUFA content in Month 3 for metal drum
	Month 6	FTT vs Chorkor	0.00003	Less in FTT	Oxidative degradation in FTT product
		FTT vs. Metal drum	0.0000000005	Less in FTT	Oxidative degradation in FTT product
		Chorkor vs. Metal Drum	0.0000000005	Less in Chorkor smoker	Higher extracted fat and PUFA content in Month 6 for metal drum

Table 6.4: Fatty acid profile of extracted fat of stored smoked-dry *Sardinella* sp. produced on the different ovens and stored for up to six montsh (presented on g/100g dry matter of smoked fish basis)

Parameter			FTT			Chorkor smoker			Metal drum oven		
			Month 0	**Month 3**	**Month 6**	**Month 0**	**Month 3**	**Month 6**	**Month 0**	**Month 3**	**Month 6**
Moisture content (g/100g of smoked fish)			$16.4^{3,6,C,M}$	$11.9^{0,6}$	$13.3^{0,3,C,M}$	$11.6^{6,F,M}$	11.7^{6}	$12.5^{0,3,F}$	$13.0^{3,F,C}$	$11.8^{0,6}$	$12.1^{3,F}$
Extracted fat (g/100 dry matter of smoked fish)			7.9^{M}	6.5^{M}	6.9^{M}	8.6^{M}	7.4^{M}	8.4^{M}	$14.2^{3,6,F,C}$	$10.7^{0,F,C}$	$10.0^{0,F,C}$
Mean SFA (g/100g dry matter of smoked)	C10:0	Capric acid	0.05	0.05	0.04	0.04	0.04	0.05	0.1	0.1	0.05
	C12:0	Lauric acid	0.1	0.06	0.06	0.1	0.05	0.1	0.1	0.1	0.1
	C14:0	Myristic acid	0.4	0.3	0.4	0.6	0.5	0.5	1.1	0.9	0.9
	C16:0	Palmitic acid	1.2	1.0	1.1	1.4	1.1	1.2	2.2	1.8	1.8
	C18:0	Stearic acid	0.3	0.3	0.3	0.4	0.3	0.3	0.5	0.5	0.5
	C20:0	Arachidic acid	0.03	0.03	0.03	0.04	0.03	0.03	0.1	0.1	0.1
	C22:0	Behenic acid	0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.03
	C24:0	Lignoceric acid	0.02	0.02	0.02	0.03	0.02	0.02	0.03	0.03	0.03
	Total SFA		$\mathbf{2.1}^{3,C,M}$	$\mathbf{1.7}^{0,C,M}$	$\mathbf{1.9}^{M}$	$\mathbf{2.5}^{3,6,F,M}$	$\mathbf{2.1}^{0,F,M}$	$\mathbf{2.1}^{0,M}$	$\mathbf{4.1}^{3,6,F,C}$	$\mathbf{3.3}^{0,F,C}$	$\mathbf{3.4}^{0,F,C}$
Mean MUFA (g/100g dry matter of smoked)	C14:1	Myristoleic acid	0.06	0.05	0.05	0.1	0.1	0.05	0.1	0.1	0.1
	C16:1	Palmitoleic acid	0.3	0.3	0.3	0.5	0.4	0.4	1.0	0.7	0.7
	C18:1c9	Oleic acid	0.2	0.2	0.2	0.3	0.2	0.3	0.5	0.3	0.3
	C18:1c11	*cis*-Vaccenic acid	0.2	0.1	0.1	0.2	0.2	0.2	0.4	0.4	0.4
	C20:1	Gondoic acid	0.02	0.01	0.01	0.02	0.01	0.02	0.04	0.02	0.02
	C22:1	Erucic acid	nd	nd	nd	0.01	nd	0.01	0.02	0.01	0.01
	C24:1	Nervonic acid	0.02	0.02	0.03	0.04	0.03	0.03	0.04	0.03	0.03
	Total MUFA		$\mathbf{0.9}^{C,M}$	$\mathbf{0.7}^{C,M}$	$\mathbf{0.8}^{M}$	$\mathbf{1.1}^{3,F,M}$	$\mathbf{0.9}^{0,F,M}$	$\mathbf{0.9}^{M}$	$\mathbf{2.1}^{3,6,F,C}$	$\mathbf{1.5}^{F,C}$	$\mathbf{1.6}^{F,C}$
Mean PUFA (g/100g dry matter of smoked)	C18:2 ω6	Linoleic acid	0.1	0.1	0.1	0.1	0.1	0.08	0.1	0.1	0.1
	C18:3 ω3	Linolenic acid	0.05	0.04	0.04	0.05	0.04	0.04	0.1	0.05	0.05
	C20:2 ω6	Eicosadienoic acid	0.1	0.04	0.04	0.1	0.05	0.05	0.1	0.1	0.1
	C20:3 ω6	Dihomogammalinolenic acid	0.01	0.01	0.01	nd	0.01	nd	0.01	0.01	0.01
	C20:4 ω6	Arachidonic acid	0.1	0.1	0.1	0.2	0.1	0.1	0.3	0.2	0.2
	C20:4 ω3	Eicosatetraenoic acid	0.01	0.01	0.01	0.02	0.01	0.01	0.03	0.02	0.02
	C20:5 ω3	Eicosapentaenoic acid	0.3	0.2	0.2	0.4	0.3	0.4	0.9	0.7	0.7
	C22:4 ω6	Docosatetraenoic acid	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
	C22:5 ω6	Docosapentaenoic acid ω6	0.05	0.04	0.04	0.05	0.04	0.04	0.06	0.05	0.05
	C22:5 ω3	Docosapentaenoic ω3	0.04	0.03	0.03	0.05	0.03	0.04	0.08	0.1	0.1
	C22:6 ω3	Docosahexaenoic acid	0.7	0.6	0.5	0.8	0.6	0.6	0.8	0.7	0.7
	Total PUFA		$\mathbf{1.4}^{3,6,C,M}$	$\mathbf{1.1}^{0,M}$	$\mathbf{1.0}^{0,C,M}$	$\mathbf{1.8}^{3,6,F,M}$	$\mathbf{1.3}^{0,M}$	$\mathbf{1.3}^{0,F,M}$	$\mathbf{2.5}^{3,6,F,C}$	$\mathbf{2.1}^{0,F,C}$	$\mathbf{2.1}^{0,F,C}$
Unidentified fatty acids (g/100g extracted fat)			0.4	0.3	0.3	0.5	0.3	0.3	0.7	0.6	0.6
Total fatty acids (g/100g of extracted fat)			$\mathbf{4.8}^{3,C,M}$	$\mathbf{3.8}^{0,C,M}$	$\mathbf{4.0}^{M}$	$\mathbf{5.8}^{3,6,F,M}$	$\mathbf{4.6}^{0,F,M}$	$\mathbf{4.7}^{0,M}$	$\mathbf{9.4}^{3,6,F,C}$	$\mathbf{7.5}^{0,F,C}$	$\mathbf{7.6}^{0,F,C}$

SFA = saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid; nd=not detected

For each fatty acid, values are means of 5 replicates. Relative standard deviations (not shown) of individual measurements were typically less than 20%.

Where used, superscripts indicate significant difference at $p<0.05$. Numerical superscripts represent a comparison among months for the same oven, and the letter superscripts for comparison among the three ovens for the same month. For the numerical superscripts, 0=different from month 0; 3=different from month 3; 6 = different from month 6. For the letter superscripts, F= different from FTT; C=different from Chorkor smoker; M = different from metal drum oven.

Table 6.5: Summary of differences in changes in PUFA levels in smoked-dried *Sardinella* sp. produced on different ovens and stored up to 6 months (assessed per dry matter of content of smoked fish)

Factor	Level	Comparison	p-value	Difference in mean PUFA level	Comment on stability of PUFAs
Same oven, different months	FTT	Month 0 vs Month 3	0.01	Less in month 3	Oxidative degradation with time
		Month 0 vs Month 6	0.003	Less in month 6	Oxidative degradation with time
		Month 3 vs Month 6	0.509	*None*	PUFAs stable
	Chorkor Smoker	Month 0 vs Month 3	0.001	*Less in month 3*	Oxidative degradation with time
		Month 0 vs Month 6	0.003	*Less in month 6*	Oxidative degradation with time
		Month 3 vs Month 6	0.747	None	PUFAs stable
	Metal Drum	Month 0 vs Month 3	0.002	*Less in month 3*	Oxidative degradation with time
		Month 0 vs Month 6	0.004	*Less in month 6*	Oxidative degradation with time
		Month 3 vs Month 6	0.941	None	PUFAs stable
Same month, different ovens	Month 0	FTT vs Chorkor	0.016	*Less in FTT*	PUFAs in FTT products less stable
		FTT vs. Metal drum	9×10^{-10}	*Less in FTT*	PUFAs in FTT products less stable
		Chorkor vs. Metal Drum	1×10^{-6}	Less in Chorkor smoler	PUFAs in Chorkor smoker products less stable and/or higher extractable fat and PUFA content in Month 3 for metal drum
	Month 3	FTT vs Chorkor	0.092	None	PUFAs stable
		FTT vs. Metal drum	7×10^{-10}	Less in FTT	Oxidative degradation in FTT product
		Chorkor vs. Metal Drum	7×10^{-8}	Less in Chorkor smoker	PUFAs in Chorkor smoker products less stable and/or higher extractable fat and PUFA content in Month 3 for metal drum
	Month 6	FTT vs Chorkor	0.018	Less in FTT	Oxidative degradation in FTT product
		FTT vs. Metal drum	1×10^{-9}	Less in FTT	Oxidative degradation in FTT product
		Chorkor vs. Metal Drum	1×10^{-6}	Less in Chorkor smoker	Higher extracted fat and PUFA content in Month 6 for metal drum

Italics = different outcome compared with results for PUFA content on extracted fat basis

6.2.4 Fatty acid profile of fish oil capsules

For the capsule oils, since FAME contents were not determined in replicates, the results for the six brands were pooled to enable statistical comparison among the treatments for discussion. Results for the individual brands are provided in Appendix 4. The pooled results were examined for normality, checked for outliers and compared by a one-way ANOVA. No significant differences (p=0.689) were found between mean PUFAs in the untreated (54 ± 2 g/100g) and stored (50 ± 1 g/100g) samples. However, the PUFA level in the thermoxidized sample (11 ± 5 g/100g) was significantly lower than in the untreated (p=0.02) and the stored oils (p=0.08), emphasizing the thermo-oxidative degradation of the compounds.

To show the effect of the epoxidation treatment on capsule oils, the FAME profile of the epoxidized Biover is presented in Table 6.6. It is noted that the MUFA content was reduced by 62% and the PUFA by 81%, owing to the greater susceptibility of the latter to oxidation (Frankel, 2012).

Table 6.6: Fatty acid profile of untreated, stored, thermoxidized and epoxidized Biover oil capsule (all data reported in g/100g of oil)

Fatty acid			Untreated	3-week storage	Thermoxidized	Epoxidized
SFA	C14:0	Myristic acid	nd	nd	nd	0.5
	C16:0	Palmitic acid	0.5	0.4	0.4	0.4
	C18:0	Stearic acid	3.3	3.0	3.1	3.0
	C20:0	Arachidic acid	0.4	0.8	0.9	0.6
	C22:0	Behenic acid	0.3	0.2	0.1	0.2
	C24:0	Lignoceric acid	0.1	0.1	0.1	n.d
	Total		**4.6**	**4.5**	**4.5**	**4.7**
MUFA	C16:1	Palmitoleic acid	0.4	0.3	0.3	0.2
	C18:1c9	Oleic acid	6.7	6.2	6.3	2.4
	C18:1c11	*cis*-Vaccenic acid	2.8	2.5	2.6	1.0
	C20:1	Gondoic acid	1.7	1.5	1.7	0.8
	C22:1	Erucic acid	1.6	1.4	1.5	0.4
	C24:1	Nervonic acid	0.3	nd	0.2	0.1
	Total		**13.5**	**11.9**	**12.6**	**4.9**
PUFA	C18:2 ω6	Linoleic acid	1.4	1.3	1.2	0.4
	C18:3 ω3	Linolenic acid	0.9	0.9	0.8	0.3
	C20:2 ω6	Eicosadienoic acid	2.1	1.9	1.6	0.5
	C20:3 ω6	Dihomogammalinolenic acid	0.1	0.2	0.1	nd
	C20:4	Arachidonic acid	2.1	1.9	1.5	0.5
	C20:5 ω3	Eicosapentaenoic acid	30.1	26.9	20.4	5.9
	C22:6 ω3	Docosahexaenoic acid	19.7	18.0	12.7	3.3
	Total		**56.6**	**51.0**	**38.3**	**11.0**
Unidentified fatty acids			12	10.1	8.5	6.2
Total fatty acid (g/100g)			**86.6**	**77.5**	**63.9**	**26.8**

nd = not detected. Values are single measurements

6.2.5 Identification of methyl EPA- and methyl DHA-derived epoxides in the in-house reference standard

The chromatograms of the epoxidized methyl EPA and methyl DHA (reference standards) are shown in Fig.6.3 and 6.4. Based on the fragmentation patterns described in Section 6.1.4.3.2 and using the mass spectra of the individual peaks, four regioisomers of methyl EPA-derived epoxides were identified, each present in their *cis* and *trans* configurations (Table 6.7). For the identification of the regioisomers, the *m/z* spectra of presumptive peaks were carefully scrutinized for characteristic ions that could distinguish one isomer from the other (Table 6.1). For example, for methyl 14,15-epoxy-5,8,11,17-eicosatetraenoate, a cleavage between carbons 15 and 16 (counting from the α-carbon, Chapter 1, Section 1.5.1) accompanied by a loss of methanol at the ester end of the molecule results in *m/z* 232 and 31. The *m/z* 232 is a characteristic ion for the compound, representing the M-31 peak. However, the, *m/z* 31 ion by itself is not useful for structural elucidation as it can also be found in other regioisomers. Methyl 8,9-epoxy-5,11,14,17-eicosatetraenoate was identified with characteristic ions including *m/z* 141 (resulting from a C7-C8 cleavage with release of *m/z* 191) and *m/z* 178 (resulting from opening of the epoxy ring by a C8-C9 split and a split between C8 and the epoxy oxygen). In this way, the four regioisomers of methyl EPA-derived epoxides were identified (Table 6.7). The *cis* and *trans* isomers were confirmed by a comparison of the mass spectra of peaks and by considering that on the CP Sil 88^{TM} FAME column, *trans* isomers elute first (Mubiru et al., 2013). Five DHA-derived epoxy regioisomers were also identified in the same way (Table 6.7).

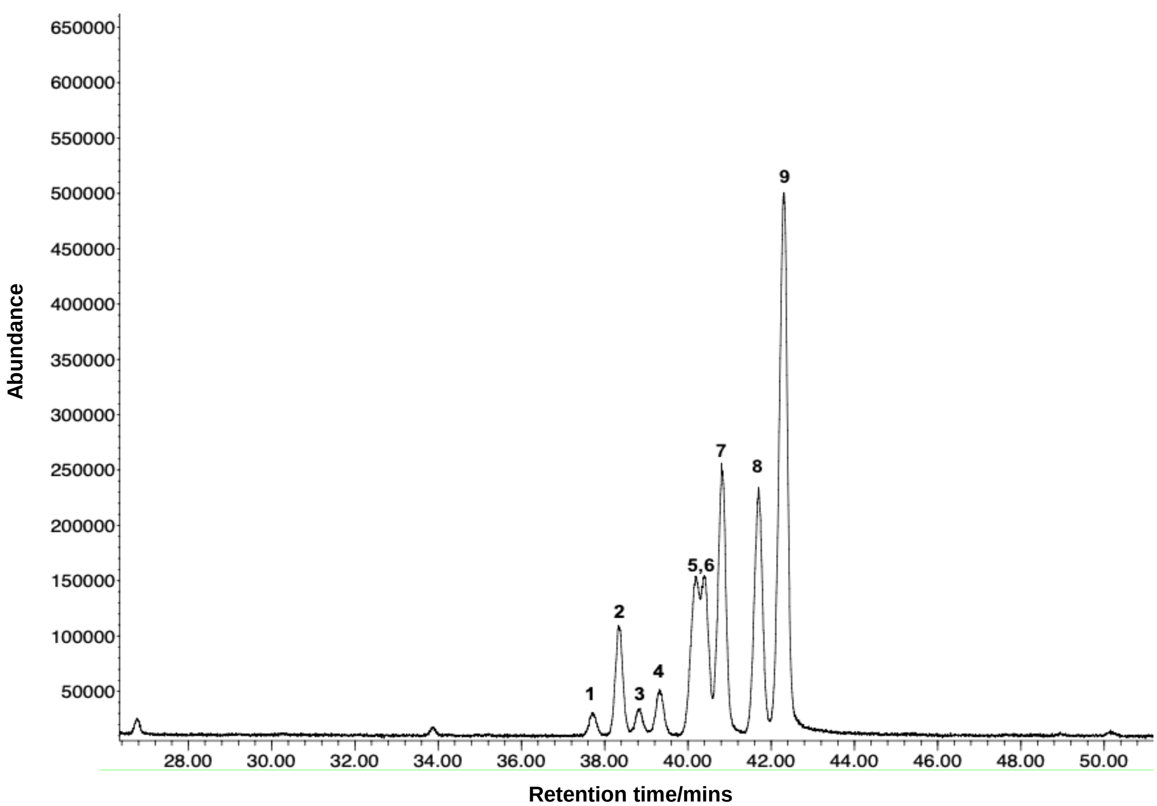


Fig 6.3: Chromatogram of epoxidized methyl EPA peaks on GC-MS coupled with a CP Sil 88^{TM} column. The identity of each numbered peak is given in Table 6.5

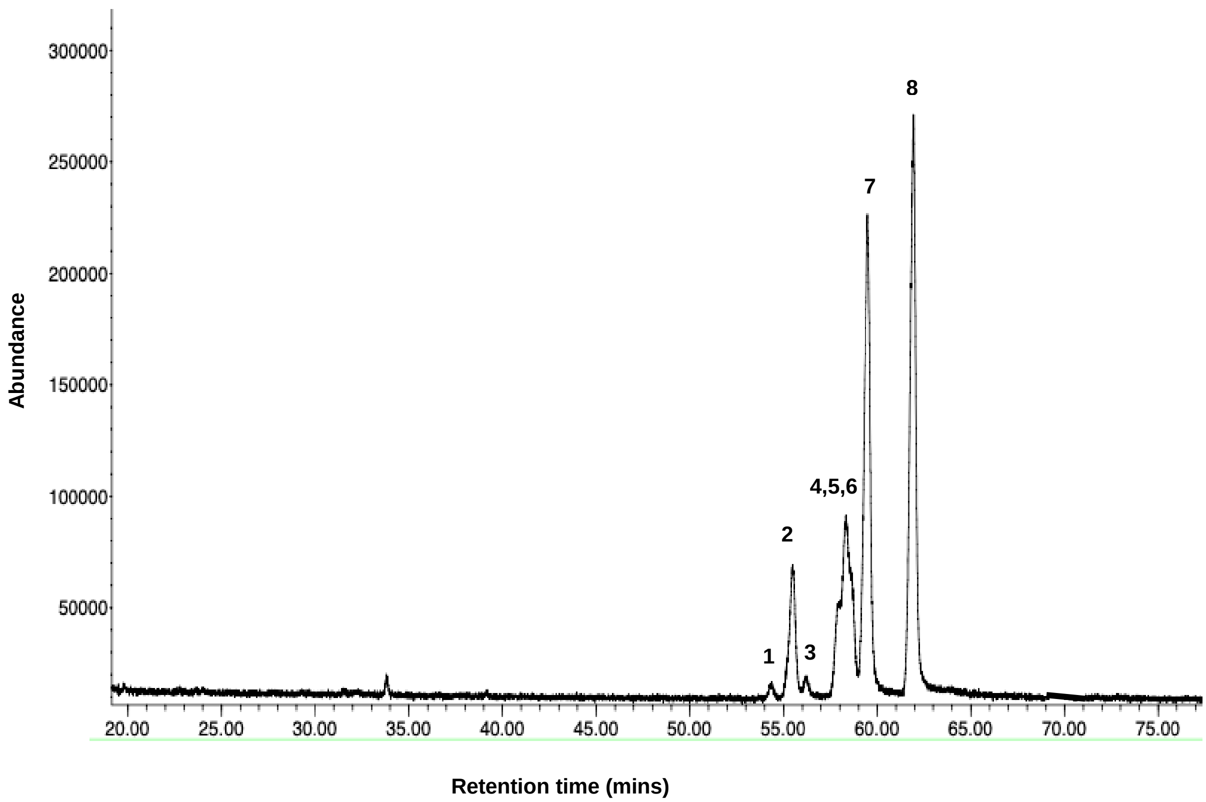


Fig. 6.4: Chromatogram of epoxidized methyl DHA on GC-MS coupled with a CP Sil 88™ column. The identity of each numbered peak is given in Table 6.5

Table 6.7: Mass spectral ions of identified derived epoxides of methyl eicosapentaenoic acid and methyl docosahexaenoic acid in extracted smoked fish fat and fish oil capsules (decapsulated)

Group of epoxides	Regioisomer	Peak	Spectral ions [m/z (relative abundance %)]
Epoxides of methyl 5,8,11,14,17-eicosapentaenoic acid	Methyl 14,15-epoxy-5,8,11,17-eicosapentaenoate	1 (trans) 2 (cis)	**232(2)**, *42(7), 69(8), 82(4),* 41(43), 67(47), 77(45), 79(82), 91(100), 93(45), 105(49), 106(2), 117(40), 119(30),131 (32),
	Methyl 11,12-epoxy-5,8,14,17-eicosapentaenoate	3 (trans) 4 (cis)	**151(6)**,*109(18)*, *122 (5)*, 41(54), 55(50), 67(69), 77(41), 79(100), 80(34), 81(45), 91(64), 95(43), 105(53), 119(33),
	Methyl 8,9-epoxy-5,11,14,17-eicosapentaenoate	5,6 (trans) 7 (cis)	**141(4)**, **152(1)**, **154(1), 170(1)**, **178(1)**, **191(3)**, *149(3)*, *162(1)*, 41(52), 55(43), 67(64), 77(36), 79(100), 80(35), 81(46), 91(63), 93(41), 105(40),
	Methyl 5,6-epoxy-8,11,14,17-eicosapentaenoate	8 (trans) 9 (cis)	**101(7)**, **112(4)**, **114(1)**, **130(5)**, *42(6), 189(2)*, *231(2)*, 55(50), 67(50), 77(40), 79(100), 91(74), 93(48), 99(32), 105(37), 117(31), 131(31)
Epoxides of methyl 4,7,10,13,16,19-docosahexaenoic acid	Methyl 16,17-epoxy-4, 7,10,13,19-docosahexaenoate	1 (trans) 2 (cis)	**111(2),** *69(4)*, *82(2)*, 44(26), 55(35), 67(51), 77(43), 78(21), 80(26), 91(100), 105(52), 131(30)
	Methyl 13,14-epoxy-4, 7,10,16,19-docosahexaenoate (co-elution)	2	**151(1),** *109(7)*, *112(1)*, 44(26), 55(35), 67(51), 77(43), 78(21), 80(26), 91(100), 105(52), 131(30)
	Methyl 10,11-epoxy-4,7,13,16,19-docosahexaenoate	4 (trans) 5 (cis)	**178(1), 190(1), 191(1)**,*149(4),162(1),* 41(60), 67(63), 77(48), 79(100), 81(32), 91(97), 93(50), 105(51), 117(46), 55(44), 131(31)
	Methyl 7,8-epoxy-4,10,13,16,19-docosahexaenoate	6 (trans) 7 (cis)	**71(3)**, **138(1)**, **140(1)**, **156(1)**, **218(1),***189(1), 202(1),* 41(49), 55(35), 67(59), 77(39), 79(100), 81(34), 91(72), 93(45), 105(39)
	Methyl 4,5-epoxy-7,10,13,16,19-docosahexaenoate	8	**98(1)**, **100(1), 116(5), 271(1),** *87(2), 229(1),* 41(45), 67(46), 77(46), 79(80), 91(100), 93(40), 105(49), 117(48), 119(33), 131(33)

Bold font: Characteristic ions; Italics: other ions from the fragmentation pattern; Normal font: Other ions present at ≥30% relative abundance
*Peak splitting

In Table 6.7, it is seen that for both methyl EPA- and methyl DHA-derived epoxides, regioisomers bearing the epoxy group closer to the omega-end of the molecule eluted first on the polar CP Sil 88™ column. Thus, it appears the polarity of these monoepoxides increased with increasing proximity of the epoxy group to the carbonyl group. This elution order is consistent with the findings of Newman et al., 2002, who found that the retention times of derived epoxides of EPA increased from the 14,15-epoxide (first to elute) through to the 5,6-epoxide (last to elute).

It is also noted that the omega-3 double bond was not epoxidized, neither for methyl EPA nor methyl DHA (witness non-detection of 17,18-derived epoxide of EPA nor 19,20-derived epoxide of DHA, Table 6.7). Newman et al., (2002) also detected all regioisomers of EPA-derived epoxides except the 17,18 regioisomer in a non-food medium (urine of hypertensive rats). It is considered that at the omega-end, the double bonds are less exposed, being buried within the cyclic geometry of the molecule and thus shielded from oxidation. A 3D structure of methyl EPA is shown in Fig. 6.5 to illustrate this point.

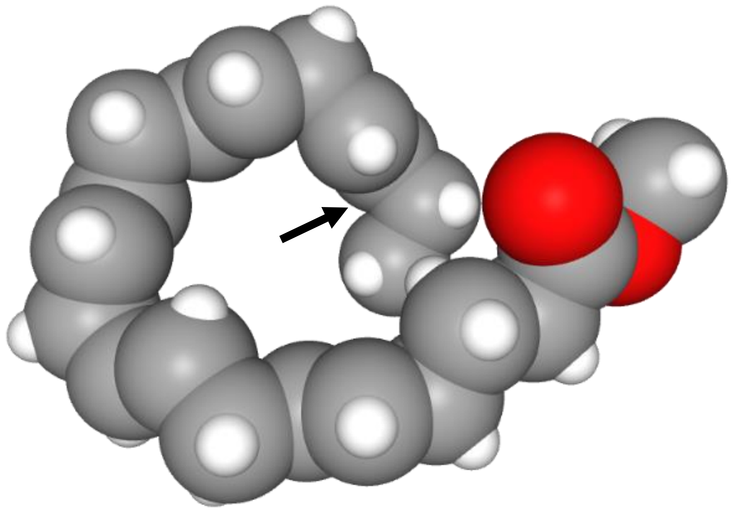


Fig. 6.5: 3D structure of methyl EPA, showing the shielding of the omega-3 double bond (arrowed) by the cyclic geometry of the compound from oxidative attack

6.2.6 Identification of EPA- and DHA-derived epoxides in smoked fish fat extract and in capsule oils

For the polar fractions of smoked fish fat extract and capsule oils, the epoxy peaks were confirmed by comparing their retention times with those of the identified peaks in the reference standards. Derived epoxides of EPA and DHA were found in all smoked fish fat extract samples. A sample GC-MS chromatogram (from FTT Month 0) is shown in Fig. 6.6A. An overlaid chromatogram of the reference standards is also shown (Fig. 6.6B) to provide a view of the location of the epoxides in relation to other peaks in the polar fraction of the smoked fish fat. From Fig. 6.6A, it is seen that the peak abundances corresponding to EPA- and DHA-derived epoxies are indeed small, highlighting the low occurrence of these epoxides (discussed later in Section 3.7).

In Figs. 6.7 and 6.8, the peaks of the reference standards are overlaid with the FTT Month 0 polar fraction to show the specific peaks corresponding to the identified regioisomers of EPA- and DHA-derived epoxides. The same is done for a sample of epoxidised capsule oil (Figs. 6.9 - 6.11)

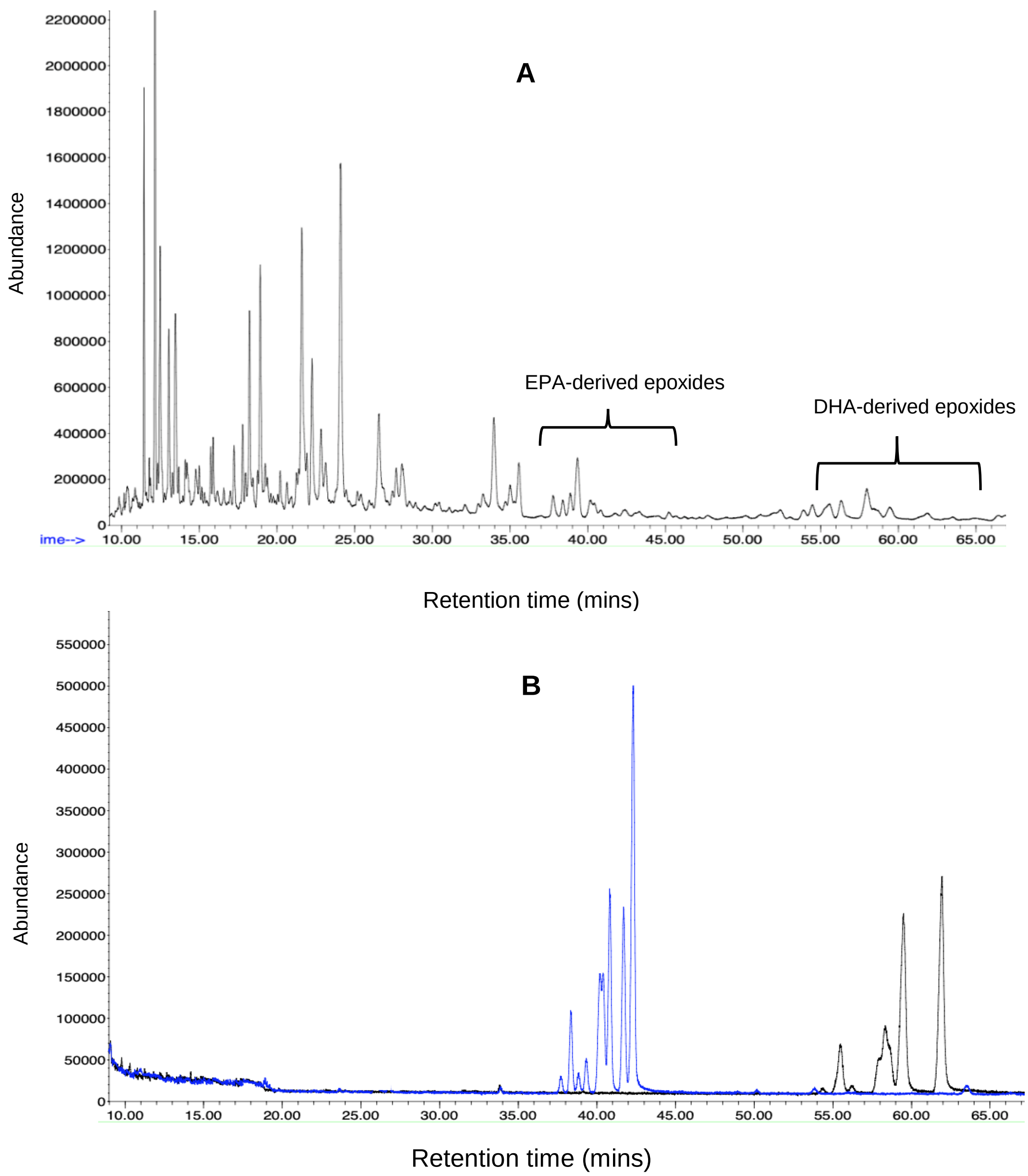


Fig. 6.6: Chromatogram of polar fraction of extracted fat of smoked fish from FTT Month 0 storage (A) and chromatogram of the reference methyl EPA- and methyl DHA-derived epoxide standards (B) on GC-MS coupled with CP Sil 88^{TM} column

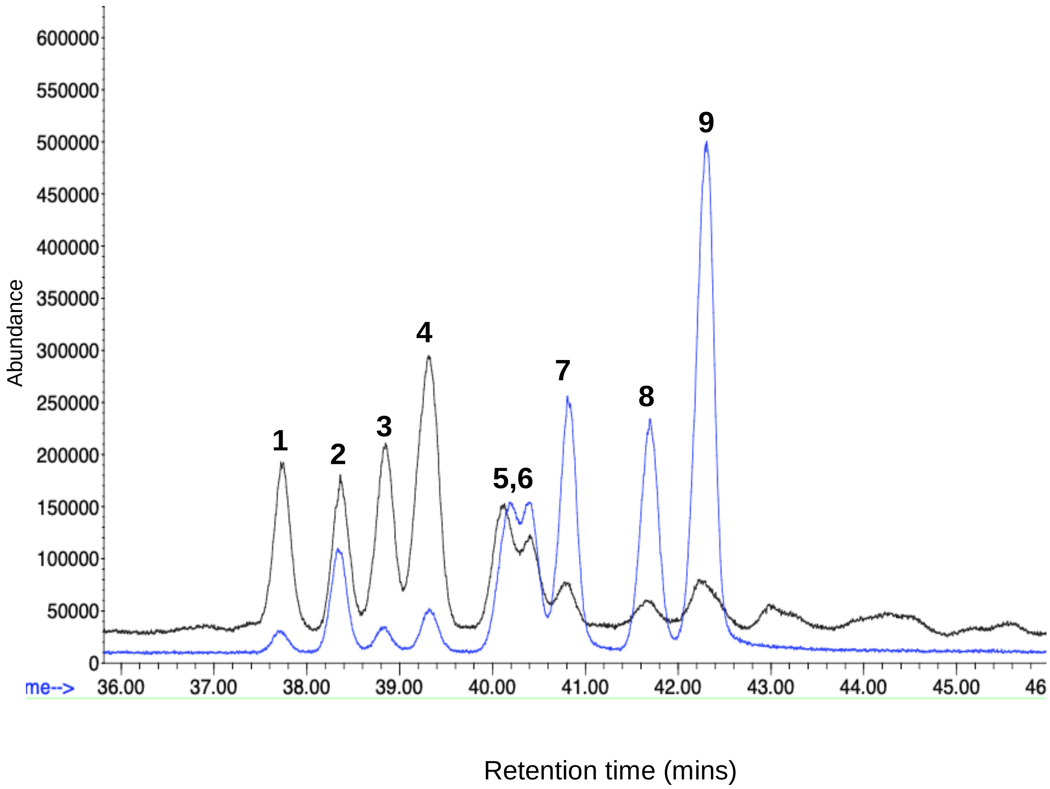


Fig. 6.7 Overlaid chromatogram of EPA-derived epoxides in extracted fat of smoked fish (FTT Month 0, black line) and the reference methyl EPA-derived standard (blue line)

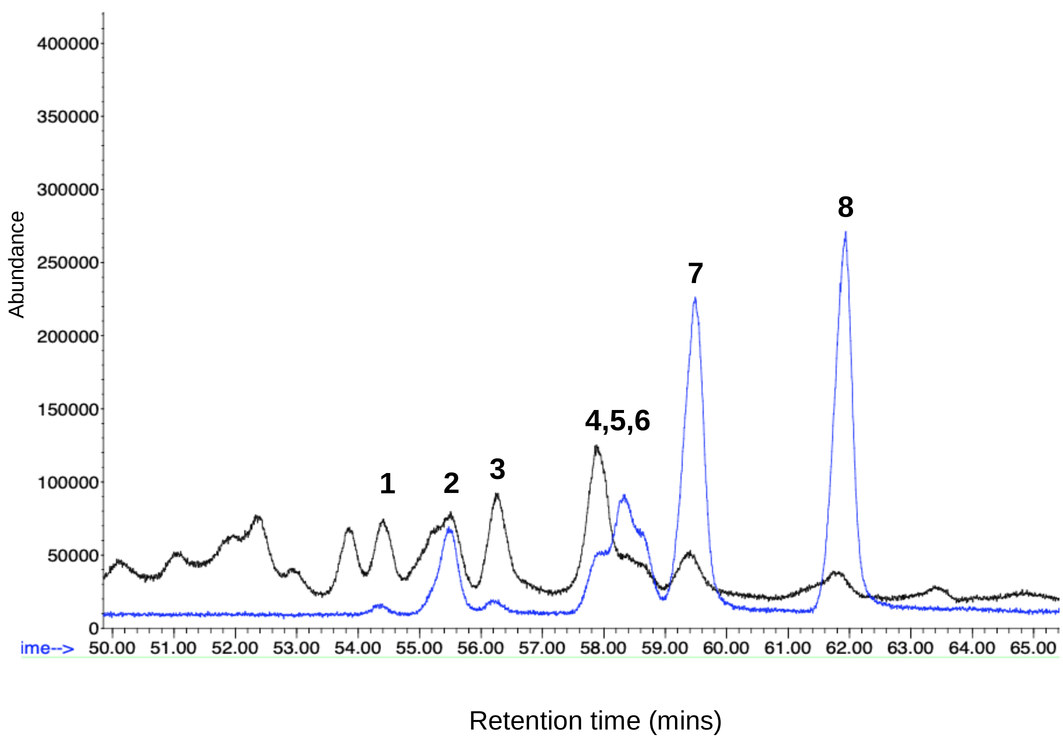
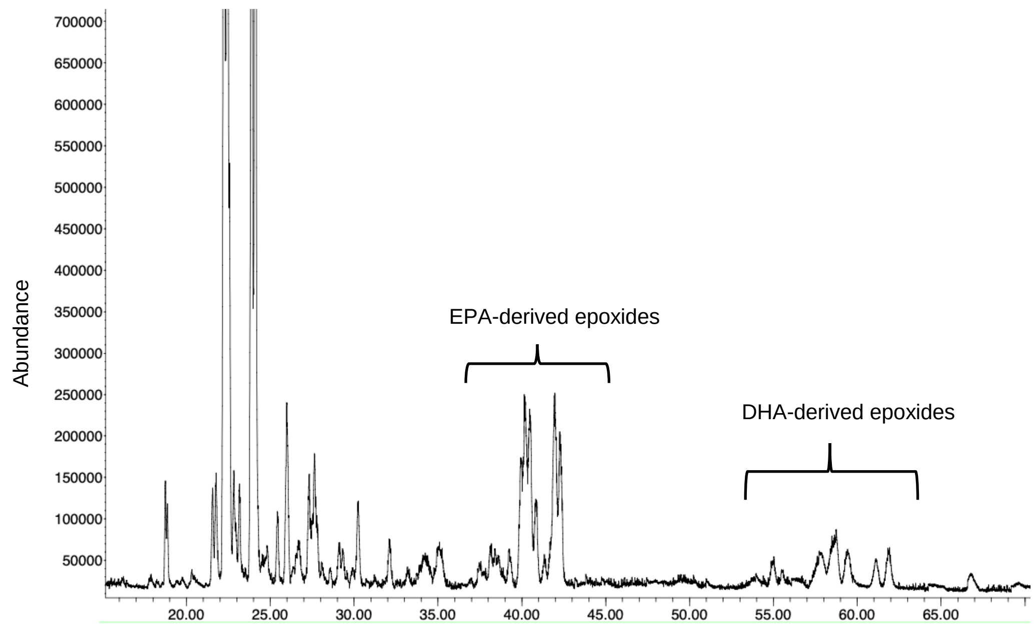
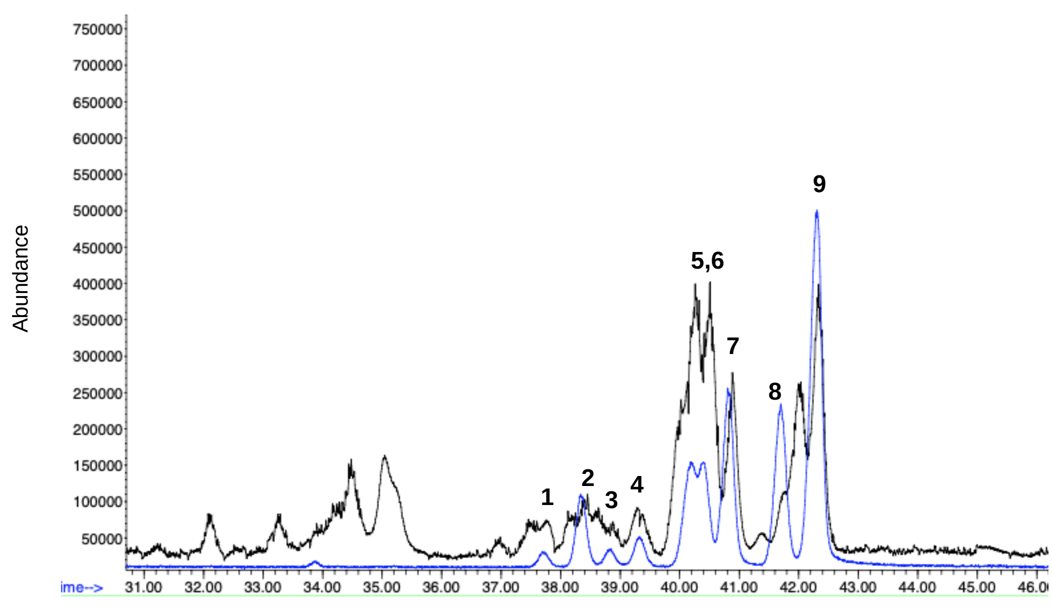


Fig. 6.8 Overlaid chromatogram of DHA-derived epoxides in extracted fat of smoked fish (FTT Month 0, black line) and the reference methyl DHA-derived standard (blue line)



Retention time (mins)

Fig. 6.9: Sample chromatogram of epoxidized fish oil capsule (Biover) on GC-MS coupled with CP Sil 88^{TM} column



Retention time (mins)

Fig. 6.10: Overlaid chromatogram of EPA-derived epoxides in epoxidized Biover capsule oil (black line) and the reference methyl EPA-derived standard (blue line)

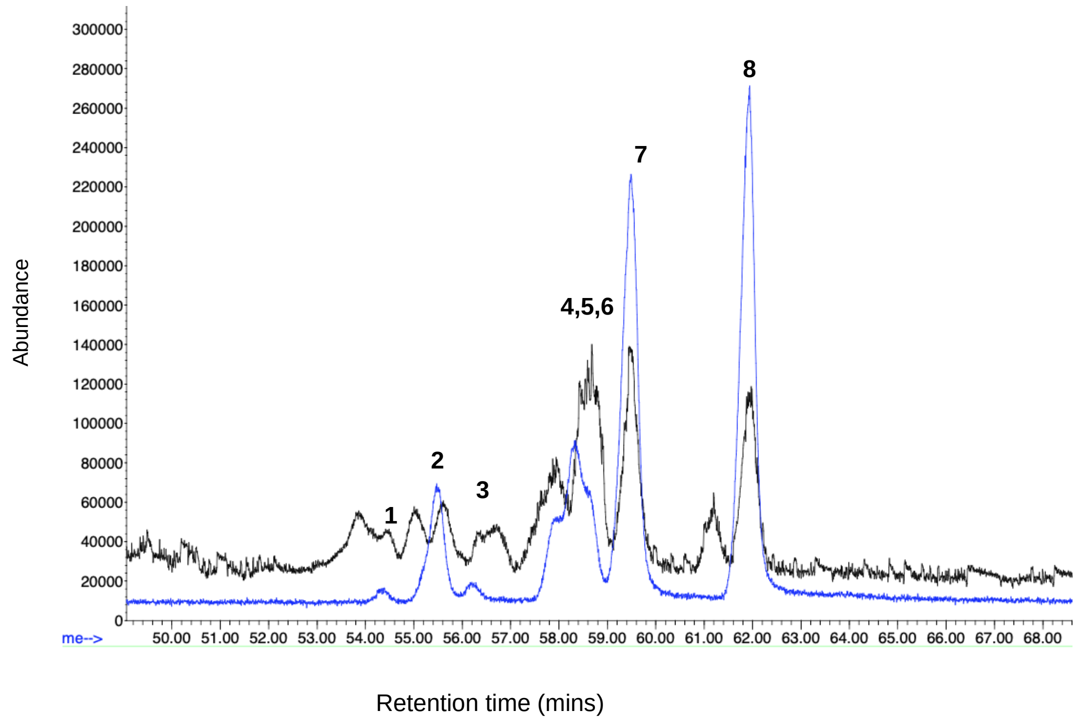


Fig. 6.11 Overlaid chromatogram of DHA-derived epoxides in extracted fat of smoked fish (FTT Month 0, black line) and the reference methyl DHA-derived standard (blue line)

It is apparent that DHA was less susceptible to epoxidation than EPA, in both the smoked fish fat extract (Fig. 6.6A) and the decapsulated fish oils (Fig. 6.9, compare derived epoxy peak abundances with others). The observation may be due to less exposure of DHA double bonds to oxidation on account of the geometry of the molecule, as similarly suggested for the omega-3 double bond (Fig. 6.5).

In Fig. 6.6A, it is seen that several peaks (occurring before the EPA-derived epoxides) remained unidentified in the polar fraction of the smoked fish fat extracts, as identification was based on peaks that could be confirmed with the reference standards. Since the polar fraction injected on the GC-MS was the epoxy/oxo fraction (extracted through a validated SPE method (Mubiru et al. (2013)), it is probable that the other unidentified peaks are either derived epoxides of other unsaturated fatty acids, or oxo-fatty acids, or a mixture of the two. To evaluate this, the potential presence of epoxides derived from oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) in the smoked fish fat extract and in the decapsulated fish oil were determined using mass spectral data from Mubiru et al., (2013). (The named fatty acids were present in the FAME profile of the smoked fish fat extracts (Table 6.2) and the decapsulated fish oils (Table 6.4)). The results are shown in Table 6.8.

Table 6.8: Identified derived epoxides of C18:1, C18:2 and C18:3 in the polar fraction of epoxidized decapsulated fish oil. The compounds were also detected in the polar fractions of the smoked fish fat extracts.

Retention Time (min)	Epoxide	Spectral ions [m/z (relative abundance%]
18.173 – 18.910 (cis) 22.35 (trans)	Methyl 9,10 epoxy 12,15 octadecadienoate	**108(5), 155(55), 185(2),** 67(37), 79(13), 93(10), 167(7)
21.504 – 22.403	Methyl 9,10 epoxyoctadecanoate	**155(100)**, **199(15)**, *281(1),* 55(95), 83(39), 97(39), 109(29), 171(17), 294(1)
22.164 – 22.455	Meth 9,10 Epoxy octadec-12-enoate	**111 (11), 151(100)**, **185(2)**, **207(1)**, *200(2), 279(1),* 55(100), 67(40), 81(32), 95(31), 109(29), 135(4), 168(4)
23.788 – 24.227	Methyl 12,13 Epoxy octadec-9-enoate	**164(11), 207(5),** *279(1),* 55(100), 74(24), 81(79), 95(53), 99(16), 123(18), 136(13), 149(10), 167(12)
20.139 – 20.417 (trans) 24.20 (cis)	Methyl 12,13 epoxy 9,15 octadecadienoate	**111(14), 207(4)**, *171(4), 189(5)*, *239(1),* 67(67), 77(11), 81(69), 83(39), 95(38), 108(9), 123(2), 147(7), *151(4)*, 161(3)

Bold: Characteristic ions for identification; italics: ions from fragmentation pattern; normal font: other ions

From Table 6.8, it is noted that the derived epoxides of C18:1, C18:2 and C18:3 were found in both the decapsulated oils and smoked fish fat extracts. It is also seen in Fig. 6.9 that the peaks corresponding to these compounds (cf. retention times) were in much higher abundances than the peaks for the derived epoxides of EPA and DHA. These show that their corresponding fatty acids were more susceptible to epoxidation than EPA and DHA. Furthermore, since the epoxides of these fatty acids (i.e. C18:1, C18:2 and C18:3) were also found in the smoked fish fat extracts, it is probable that the total amount of epoxides produced during the smoking process and storage may be much higher than the sum of EPA- and DHA-derived epoxides.

6.2.7 Semi-quantitative estimation of EPA- and DHA-derived epoxide content as indication of lipid oxidation

The semi-quantitative estimations of the amounts of EPA- and DHA-derived epoxides calculated based on the relative peak areas of the epoxides are summarized in Tables 6.9 and 6.10. From Table 6.9, it is seen that at Month 0, epoxides derived from EPA and DHA were present in all extracted smoked fish fat samples but at low amounts (generally less than 15%, semi-quantitatively determined). The low amounts suggest that the other oxidized species are probably more important (accounting for up to 85% of the total peak area) than the epoxides derived from EPA and DHA. Furthermore, it is noted that no clear increasing trend in the relative percentages of these compounds (i.e. derived epoxides of EPA and DHA) is observed over the storage period. It is critical to note that the power of these semi-quantitative estimations is indeed low, and that more rigorous quantitative estimations will be required to ascertain the precise extents to which these differences occur.

As expected, the EPA+DHA derived epoxides in the epoxidized fish capsule oils were up to two times the amounts in the smoked fish fat extracts. Since the capsule oils were deliberately epoxidized, it is expected that the other peaks were derived epoxides of other unsaturated fatty acids (see Table 6.6), which then would account for up to 70% of the peaks. This further highlights the probable occurrence of epoxides from other unsaturated fatty acids in the smoked fish fat extracts, beyond those identified in Table 6.6.

From Table 6.10, it is seen that the dominant EPA-derived epoxide in all the smoked fish samples was methyl *cis*-11,12-epoxy-5,8,14,17-eicosatetraenoate, whereas the dominant DHA-derived epoxide was methyl *trans*-7,8-epoxy-4,10,13,16,19-docosapentaenoate.

Table 6.9: Relative occurrence of EPA and DHA epoxides in the extracted fat of stored smoked-dry *Sardinella* sp. from different ovens, and in treated fish oil capsules

Sample	Oven/brand	Treatment	EPA Epoxides (%)	DHA Epoxides (%)	(EPA+DHA) Epoxides (%)
Smoked fish samples	FTT	Month 0	3.7	4.2	7.9
		Month 3	5.3	7.7	12.9
		Month 6	4.6	5.2	9.7
	Chorkor smoker	Month 0	6.3	11.7	18.0
		Month 3	5.8	5.2	11.0
		Month 6	6.4	5.6	12.0
	Metal drum oven	Month 0	8.8	5.6	14.4
		Month 3	7.0	4.5	11.5
		Month 6	9.0	5.61	14.6
Fish oil capsules	Biover	Epoxidized	22.2	11.0	33.2
		Thermoxidized	3.9	nd	3.9
	Boni	Epoxidized	22.2	11.0	33.3
		Thermoxidized	12.4	1.7	14.2
	Lucovitaal	Epoxidized	13.4	6.0	19.4
		Thermoxidized	15.1	1.6	16.7
	Vitarmonyl	Epoxidized	24.6	10.3	34.9
		Thermoxidized	nd	nd	nd
	Kruidvat	Epoxidized	2.9	0.8	3.7
		Thermoxidized	2.7	0.6	3.3

nd = not detected

Table 6.10 Relative occurrence of regioisomers of methyl EPA- and methyl DHA-derived epoxides in the extracted fat of stored smoked-dry *Sardinella* sp., calculated as percentage peak area of each isomer relative to the total peak area of the corresponding group of epoxides

			Percentage area (%)								
			FTT			Chorkor			Metal		
Group of epoxides	**Regioisomer**	**Configuration**	**Month 0**	**Month 3**	**Month 6**	**Month 0**	**Month 3**	**Month 6**	**Month 0**	**Month 3**	**Month 6**
Derived epoxides of methyl 5,8,11,14,17-eicosapentaenoic acid	Methyl 14,15-epoxy-5,8,11,17-eicosatetraenoate	Trans	14.5	17.5	17.2	15.3	15.5	16.6	16.6	16.7	16.6
		Cis	8.8	12.3	12.2	11.0	10.7	11.9	12.0	12.6	12.5
	Methyl 11,12-epoxy-5,8,14,17-eicosatetraenoate	Tran	9.6	12.7	10.6	13.0	10.1	11.2	13.1	11.9	11.6
		Cis	39.2	29.9	34.4	31.7	37.6	35.2	28.3	33.5	34.2
	Methyl 8,9-epoxy-5,11,14,17-eicosatetraenoate	Trans	17.0	16.6	16.6	18.8	17.1	16.0	18.7	16.6	16.2
		Cis	2.4	2.7	2.0	2.1	1.7	1.6	2.7	2.1	2.4
	Methyl 5,6-epoxy-8,11,14,17-eicosatetraenoate	Trans	2.5	2.9	2.6	2.4	2.1	2.1	2.3	2.6	2.4
		Cis	5.9	5.4	4.3	5.6	5.2	5.3	6.2	4.0	4.1
Derived epoxides of methyl 4,7,10,13,16,19-docosahexaenoic acid	Methyl 16,17-epoxy-4, 7,10,13,19-docosapentaenoate	Trans	8.2	10.7	11.0	5.3	10.6	11.3	8.2	10.0	10.3
		Cis	17.6	18.7	19.8	10.9	19.6	19.6	20.4	19.5	20.2
	Methyl 13,14-epoxy-4, 7,10,16,19-docosapentaenoate	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Methyl 10,11-epoxy-4,7,13,16,19-docosapentaenoate	Trans	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
		Cis	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Methyl 7,8-epoxy-4,10,13,16,19-docosapentaenoate	Trans	38.6	35.9	39.5	33.2	38.4	39.0	37.1	39.5	38.4
		Cis	10.9	6.3	7.7	21.5	7.8	7.3	8.0	8.1	7.5
	Methyl 4,5-epoxy-7,10,13,16,19-docosapentaenoate	-	8.9	5.7	6.9	21.5	7.8	7.3	8.2	7.2	7.5

nd=not detected in the mass spectra

6.3 CONCLUSION

The exploratory character of the study is quite patent in the results obtained. Preliminarily, it can be suggested that, on the basis of the FAME profile of the extracted fat from smoked fish processed on the assessed ovens, lipids in products of the Chorkor smoker and the metal drum oven seem to be less prone to oxidation than those in FTT products. The smoking process and traditional storage conditions have singular and combined effects on the initiation and potential progressive oxidative degradation of the PUFAs in the products. Although determined semi-quantitatively, evidence gathered suggests that derived-epoxides of methyl EPA and methyl DHA may not be suitable indicators of oxidation in (smoked) fish oil. They are present in low amounts (semi-quantitatively determined) and do not show a clear increasing trend over time. Further evaluation of the occurrence of epoxides derived from other unsaturated fatty acids may enable a more thorough assessment of the suitability of epoxides in general as indicators of oxidation in fish lipids. Furthermore, determination of other indices of oxidation (such as hexanal content) is recommended, as this may provide some insights into, and quantitative estimates of, the extent of oxidation in the lipids of smoked fish, as influenced by processing and storage.

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Up to this chapter, hazards in traditionally smoked fish in Ghana have been profiled and the most relevant in the context identified as PAHs; the use of traditional ovens has been shown to be the key driver of product contamination and subsequent consumer exposure to the hazard, and the FTT has been verified as technically efficacious for the PAH problem. It has also been shown that consumer acceptance of FTT products may not be a challenge, and that FTT products, if stored by the traditional approach, could have diminished nutritional benefits and potentially pose risks to health due to oxidation of (poly)unsaturated fatty acids. In the next chapter, these findings will be combined with socioeconomic considerations to provide a multi-criteria basis for identifying appropriate trade-offs in the adoption and use of the FTT.

CHAPTER 7: MULTICRITERIA DECISION ANALYSIS FOR THE SELECTION OF AN INTERVENTION TO REDUCE EXPOSURE TO PAHs IN SMOKED FISH IN GHANA

ABSTRACT

A multicriteria decision analysis (MCDA) approach was applied to evaluate two potential interventions for reducing consumer exposure to PAHs in smoked fish in Ghana. The interventions considered were the FTT oven (technical intervention) and consumer-end product treatment (removing the skin of smoked fish during food preparation to reduce the PAH contamination of the product). The two options were evaluated along with the Chorkor smoker (current oven for fish smoking and main contributor to PAH contamination in smoked fish). The considered evaluation criteria were food safety (ability of the intervention to reduce the PAH4 levels in smoked fish), cost (upfront financial investment), sustainable use of intervention (potential for long-term use), consumer acceptance of products from the intervention, support for environmental sustainability, and occupational hazard exposure for processors. Quantitative data from previous chapters of this thesis were combined with evidence from literature to score the interventions on each criterion. Different weights were assigned to the criteria in three computational scenarios to reflect different hypothetical stakeholder values and preferences. In Scenario I, all criteria were considered equally important to the decision maker. In Scenarios II and III, public health and economic viability were prioritized, respectively. An outranking method was then used to select the better performing intervention. The Visual PROMETHEE® software was used for the analysis. Across all scenarios, the FTT oven was ranked as the better intervention, showing greater strength than the consumer-end product treatment in all criteria except cost. In all scenarios, low cost was the only strength of the latter intervention. The Chorkor smoker (current situation) also ranked higher than the consumer-end treatment in all three scenarios. However, it underperformed in food safety, occupational hazard exposure and environmental sustainability. For the specific rankings, in Scenario I and II, the FTT ranked as 1, followed by the Chorkor smoker (rank 2) and the consumer-end product treatment (rank 3). In Scenario III (prioritizing economic viability, defined in the limited sense of upfront investment, consumer acceptance of products and sustained use of intervention), the Chorkor smoker ranked higher (rank 1) than the FTT (rank 2), however, it retained its weaknesses in food safety, occupational hazard exposure and environmental sustainability. The findings suggest that, within the limits of the assessment

parameters considered and the decision goal, the use of the FTT oven would be better than the consumer-end product treatment as an intervention for reducing consumer exposure to PAHs in smoked fish. However, it will demand a significant upfront financial investment.

Keywords: MCDA; smoking ovens; food safety; public health intervention

7.0 INTRODUCTION

Guaranteeing food safety is an important pillar of food security. It requires consideration of multiple factors that, if taken individually, may seem consequential but which, taken in context, may differ in their relevance to particular decision goals. For example, in selecting an intervention to reduce the occurrence of a hazard in a food product, to what extent should institutional/national budgets, consumer acceptance of the product concerned and/or the livelihoods of food business operators influence the decision? Which of those factors should be prioritized and on what basis? To be effective, addressing issues of that nature that are affected by several factors to varying degrees requires the application of multicriteria decision analysis (MCDA) (Garre et al., 2020).

MCDA is a process for identifying the best option among multiple, potentially conflicting decision alternatives (FAO, 2017; Garre et al., 2020). It is a rather new concept in food safety but has a long track record in medical, nutritional and environmental decision-making processes. It is recommended by the FAO as an evidence-based, rigorous and transparent process for food safety governance decisions (FAO, 2017) and has been applied to develop a framework for prioritizing foodborne pathogens in poultry meat in Canada (Ruzante et al., 2010), prioritizing emerging zoonoses in the Netherlands (Garre et al., 2020) and to simultaneously evaluate biological and chemical hazards related to emerging dietary practices in France (Eygue et al., 2020). Fazil et al. (2008) demonstrated its usefulness in selecting food safety interventions while balancing several criteria. Van der Fels-Klerx et al. (2018) also emphasized the potential of MCDA for combining different types of information (i.e. quantitative and qualitative) in ranking decision alternatives.

Concerning the safety of smoked fish in Ghana, it was demonstrated in Chapter 2 that PAHs are the principal hazards in the products and that their occurrence is due primarily to the smoking oven used. It may seem natural, then, to consider an oven intervention as the solution for the food safety problem. However, several factors – among them technological, economic, social and environmental – may have a bearing on the quality of that decision. For example, in some African countries, sustainable use of the FTT was threatened by such social factors as communal ownership of the oven and rationed use

(Mindjimba and Tiotsop, 2018; see Chapter 8). Appropriately considering each factor in context is fundamental for arriving at an optimal compromise decision. That is the crux of MCDA (Brans and Mareschal, 2005; Fazil et al., 2008).

Although it has been demonstrated that the FTT is technically viable for producing smoked fish with low and acceptable PAH levels (Chapter 3), adoption of the innovation informed solely by that basis may hurt other interests. Mindjimba et al. (2019), for instance, warned that the access of FTT products to the (more economically rewarding) export markets could result in overexploitation of fishery resources in low- and middle-income countries (LMICs), as fishers harvest larger volumes at higher frequencies in a bid to cash in on the export opportunities. This situation may endanger domestic food security. Furthermore, if the receptivity of food business operators (fish processors) to the innovation is not evaluated, the oven, upon introduction, may not enjoy sustained use, thereby causing financial losses to implementing agencies. As mentioned in Chapter 1, the Altona oven, for example, was rejected by countries that considered alien and culturally unacceptable the feature of skewing fish through the eyes for processing (Nerquaye-Tetteh, 1999; Peñarubia et al., 2017).

The response of consumers in particular contexts to products of the oven is also worth considering. In Burkina Faso, Côte d'Ivoire, the Democratic Republic of the Congo and Tanzania, some consumers did not readily accept FTT products, considering them pale in colour compared to products of the traditional ovens (Mindjimba and Tiotsop, 2018). In contrast, in Chapter 5, it was shown that in Ghana, consumer response to FTT products did not differ from that of the Chorkor smoker.

In this chapter, the MCDA approach was used to evaluate two potential interventions to reduce the high PAH exposure in smoked fish in Ghana (reported in Chapter 4). The interventions considered were the adoption and use of the FTT (technical intervention), and consumer food preparation treatments aimed at reducing PAH contamination (e.g. removing the skin of smoked fish during food preparation). These two options were evaluated alongside the Chorkor smoker (current fish smoking oven and main contributor to PAH contamination in smoked fish). It was envisaged that the study outcome would provide preliminary, multi-factorial evidence to support policy decision-making on smoked fish safety in Ghana, as required in modern food safety governance.

7.1 METHODOLOGY

The approach used consisted of five steps, namely, definition of the food safety problem, identification of potential (risk) management options, selection of criteria for evaluating the options, gathering evidence to evaluate the performance of each option on the selected criteria, and choosing the best option (FAO, 2017).

7.1.1 Definition of the food safety decision problem

The food safety decision problem was defined as *the selection of an appropriate intervention to reduce consumer exposure to PAHs in smoked fish in Ghana*.

7.1.2 Identification and selection of intervention options

As discussed in Chapters 3 and 4, PAHs in smoked fish occur principally as process contaminants. Therefore, the option for addressing their occurrence is either the use of smoking ovens/techniques that control their occurrence at the processing step (i.e. technical intervention), or the application of food preparation practices downstream (i.e. intervention at the consumer-end) to reduce the contamination after it has occurred. Thus, two intervention options were considered as follows:

i. *Use of the FTT oven*: The technical viability of the FTT to reduce PAH contamination and subsequent consumer exposure to the hazard has been demonstrated (Chapters 3 and 4). That efficacy is now considered with other criteria to evaluate the suitability of the innovation as a solution to the defined problem.

ii. *Consumer-end product treatment*: As shown in Chapter 4, the PAH level in smoked fish is significantly reduced by food preparation practices such as removal of the skin of the product (up to 87% reduction) and washing products in warm (60^{o}C) water (up to 86.5% reduction, Mahugija and Njale, 2018). It is considered that use of this intervention presupposes continued use of the traditional ovens.

These two intervention options were assessed along with the use of the Chorkor smoker to show how the status quo also performs on the assessment criteria. The metal drum oven was not assessed since it is used less in Ghana and, in principle, has been replaced by the Chorkor smoker for reasons of low fuel efficiency, low throughput and high operational drudgery, among other challenges (see Chapter 1).

7.1.3 Identification and selection of decision criteria

The decision criteria are the parameters against which the interventions are assessed (FAO, 2017). According the FAO (2017), criteria for MCDA in food safety are selected based on the specific needs and resources of the context considered, as viewed by the decision maker. Thus, the criteria reflect the value judgments of the decision maker vis-à-vis the problem on table. For the present decision problem, the criteria used and the basis of their selection are explained below:

i. *Food safety*: This measured the extent to which each intervention option reduces PAH levels in products. The concentration of PAH4 in products from each intervention was used as the metric. This metric was selected for food safety considering that, as demonstrated in Chapter 2, PAHs are the principal food safety hazards in traditionally smoked fish in Ghana. The first multi-centre sub-Saharan Africa Total Diet Study on PAHs identified smoked fish as the food item most contaminated with the hazard (Ingenbleek et al., 2019). Codex also has several normative instruments specifically addressing that hazard-food pair (CODEX STAN 311 – 2013; CAC/RP 68 – 2009), thereby underscoring its food safety relevance.

ii. *Cost*: This is a fundamental consideration in the selection of (food safety) interventions (Fazil et al., 2008). In this study, the cost (in US dollars) criterion measured the upfront financial investment required for each intervention.

iii. *Exposure to occupational hazards*: During (traditional) fish smoking, processors are exposed to several occupational hazards, chief of which is the complex mix of chemicals in the wood smoke, which have been epidemiologically linked to morbidities such as inflammatory lung conditions (Gordon et al., 2014), chronic obstructive pulmonary disease (Assad et al., 2015), lung cancer and cardiac events (Bruce et al., 2015). The risks for these conditions are doubled for women in LMICs (Bruce et al., 2015). Therefore, it was considered that an effective oven intervention for food safety must also support decent work (United Nations Sustainable Development Goal 8) for the processors (Peñarubia et al., 2017), hence the inclusion of this criterion.

iv. *Consumer acceptance of products*: As discussed in Chapter 5, consumer acceptance is pivotal to the adoption of oven interventions, as it determines the bottom line for processors. In addition, for food security, the cultural habits and food quality preferences of populations should not be ignored.

v. *Sustainable use of intervention*: This criterion was included to measure the continued (long-term) use of the intervention following its introduction. Mindjimba and Tiotsop (2018) studied the adoption and sustainable use of the FTT in Cameroun, Côte d'Ivoire and Tanzania and reported a readiness of processors to adopt the oven. However, sustained use was threatened by non-price differentiation between products of the FTT and those from the traditional ovens, although the former has an added value (safer).

vi. *Environmental sustainability*: This criterion measured the potential contribution of each intervention to environmental sustainability in respect of the kind and quantity of fuel used in its operation.

7.1.4 Evidence for evaluating criteria and assigning scores

In MCDA, it is required that each intervention option is evaluated against the selected criteria based on as much evidence as possible (Fazil et al., 2008). Typically, the evidence takes the form of research findings and/or expert opinion (FAO, 2017). For this study, the availability of evidence was generally limited, considering the traditional nature of the processing methods, the fairly recent introduction of the FTT, and the general research data paucity in the South. However, some information was obtained from the available literature and combined with data from previous chapters of this study to evaluate the interventions.

7.1.4.1 Evidence for the food safety criterion

For this criterion, the mean PAH4 concentrations reported in Chapters 3 and 4 for smoked-dry *Sardinella* sp. were used. The reported values for the FTT and the Chorkor smoker were 2.2 µg/kg and 394.5 µg/kg (Chapter 3, Table 3.1), whereas the value for the consumer-end product treatment (descaling) was 78.20 µg/kg (Chapter 4, Table 4.4).

7.1.4.2 Evidence for the cost criterion

The construction cost of an FTT ranges from USD 381 to 1,668 depending on the type and size of the first-generation improved oven upon which it is based (Mindjimba et al., 2019; Zelasney et al., 2020). As explained in Chapter 1 (Section 1.3.3.1), the characteristic components of the FTT (i.e. ember furnace, the fat collector and the indirect smoke generator) can be retrofitted to an existing first-generation improved oven. A full construction would thus attract a cost closer to the upper limit. The maximum value of the range (USD 1,668) represents an FTT built on the Chorkor base. Given the prominence of the Chorkor smoker in Ghana, it was considered that this model of FTT would be the most patronized. Hence, its cost (USD 1,668) was used as the reference cost for the FTT.

For the Chorkor smoker, typical construction costs of USD 300 – 350 have been reported (Towers, 2014; Mindjimba et al., 2019). The upper value of USD 350 was used, to represent a “worst case” (most expensive) situation. For the consumer-end product treatment, it was considered that the smoked fish to be treated that way would have been invariably processed on the Chorkor smoker. Since no *additional* financial inputs would be required to remove the skin of smoked fish, this intervention option was considered to have the same cost as the Chorkor smoker.

Although it could be argued that the consumer-end product treatment would also require consumer education (e.g. information campaigns from the public health authority and/or non-profit organisations on how and why to remove the skin of smoked fish) and thus have a cost element, such education/information campaign should ordinarily be part of any intervention and was thus not monetised uniquely for any one option. Furthermore, although other factors such as oven maintenance may have been useful in this criterion, data were not available and so this was not included.

7.1.4.3 Evidence and scoring for occupational hazard exposure criterion

Scoring for this criterion was based on the reported extent of smoke release during the use of the ovens and their associated carbon monoxide (CO) contents. The Chorkor smoker is generally known to generate excessive amounts of smoke during use (Ndiaye et al., 2015). Anoh et al. (2017) reported (qualitatively) a marked reduction in exposure to wood smoke among processors using the FTT in Cote d'Ivoire. This was also observed during the smoking sessions for this study (Fig. 7.1). The difference is ostensibly due to the different fuels used.

Fig. 7.1: Comparison of smoke exposure during the use of the FTT (left) and the Chorkor smoker (right)

Other studies have also reported elevated exposure to hazardous components in wood smoke from household stoves that use fuelwood as energy source (Gordon et al., 2014; Assad et al., 2015; Bruce et al., 2015; Quansah el al., 2017). Anoh et al., (2017) quantified CO in released smoke during the use of the FTT and the Chorkor smoker as 50mg/m^3 and 350mg/m^3, respectively. Those figures were thus used. As was done for the cost criterion, the consumer-end treatment was assigned the same value as the Chorkor smoker.

7.1.4.4 Evidence and scoring for consumer acceptance of products

For this criterion, data from Chapter 5 (sensory evaluation of FTT vs. Chorkor smoker products) was used for assessing the intervention options. Since no significant differences were found in the overall liking scores of products from the two ovens (p=0.393), the same score (3 = high) was assigned to both FTT and the Chorkor smoker. For the consumer-end product treatment, due to unavailability of data, a mid-ground score of 2 (medium acceptance) was assigned to accommodate uncertainties in consumer response to this intervention. It is also noteworthy, as mentioned in Chapter 4, that the practice of removing the skin of smoked fish is not common in Ghana, and that very few fish species can be treated that way when smoked. Considerable effort may therefore be needed to garner wholesale acceptance of the practice.

7.1.4.5 Evidence and scoring for sustainable use of intervention

As defined earlier, the sustainable use of intervention criterion refers to the continued, long-term use of an intervention following its introduction. For the sake of food security and livelihood support, it is imperative that the selected intervention be acceptable to fish processors for continued use. From the report of Mindjimba and Tiotsop (2018) (discontinued use of the FTT in some countries due to non-price differentiation of products), it was considered that addressing the business environment challenge could enhance the sustainable use of the FTT. Hence, a score of 2 (medium) was assigned to the FTT. The Chorkor smoker already enjoys widespread, continued use across Africa, hence it was assigned a score of 3 (high). Consumer-end treatment was assigned a conservative score of 1 (low), due to challenges likely to be encountered in the sustained practice of the intervention. Aboud and Singla (2012) reported the peculiar difficulties faced in sustaining behaviour change in developing countries, citing, for example, a mistrust in the urgency of change in habits when communities have managed to stay alive for years without such changes.

7.1.4.6 Evidence and scoring for environmental sustainability

This criterion was measured based on the fuel demand of the ovens. A fuel consumption of 0.25 kg charcoal/kg fish has been reported for the FTT (Ndiaye et al., 2015), and 2.08 kg fuelwood/kg fish for the Chorkor smoker (Olokor, 2003). In traditional charcoal production in LMICs, the estimated yield is 25% (Girard, 2002). Thus, the 0.25 kg charcoal/kg of fish for the FTT translates to 1 kg of wood/kg of fish. Hence, the FTT uses *at least* 50% less wood per unit of fish smoked than the Chorkor smoker (not considering dry matter contents of the wet wood and the dried fuelwood).

The FTT is also reported to emit less greenhouse gases than the Chorkor (Ndiaye et al., 2015; Anoh et al., 2017). Based on these, the FTT was considered more environmentally sustainable and was assigned a score of 2 (medium support for environmental sustainability), and the Chorkor smoker and the consumer level-treatment assigned a score of 1 (low support for environmental sustainability).

7.1.5 Criteria weights and ranking scenarios

To reflect situations where criteria may have varying importance to decision makers, weights were assigned to each criterion by an outranking approach. In assigning the weights, it would have been ideal to consult different stakeholders (e.g. fish processors, consumer representatives, food safety risk managers, etc.) to solicit their input on how they would weigh the criteria. However, due to time constraints, this could not be done. Weights were therefore assigned by the author based on knowledge of, and experience in, the context.

Three scenarios were considered as follows:

i. *Scenario I:* In this scenario, all six criteria were considered equally important and were assigned equal weights (16.67% each).

ii. *Scenario II:* In this scenario, the perspective of a stakeholder/decision maker who prioritizes public health was assumed. Therefore, food safety and occupational exposure to smoke were considered the most important criteria and were assigned equal weights of 30% each. The remaining four criteria were assigned equal weights of 10% each.

iii. *Scenario III:* This scenario prioritized the economic viability of the intervention options. Hence, the cost of intervention, consumer acceptance and sustainable use of intervention were assigned weights of 30% each. The remaining 10% was divided equally among the remaining three criteria (i.e. 3.33% each).

In each scenario, the sum of assigned weights was 100%.

The Visual PROMETHEE® software allows setting of the best value (ideal situation) for each criterion. This requires the decision maker to indicate desirable intervention performances (Fazil et al., 2008). For example, for the cost criterion, the best intervention should have the *minimum* value/score possible, whereas for consumer acceptance, the best intervention should have the *maximum* value/score possible. The software was thus instructed to minimize the cost, PAH4 impact and exposure to occupational hazards, and maximize all other criteria in its computations.

The criteria and their associated metric values/scores and sources of evidence are summarized in Table 7.1. Assigned scores, weights and ranking scenarios are also summarized in Table 7.2. The information in Table 7.2 was entered into the Visual PROMETHEE® software and the outputs discussed.

Table 7.1: Selected criteria and associated metrics, applied scoring scale and data sources for evaluating interventions for reducing PAH exposure in smoked fish in Ghana

Criteria	Metric	Decision objective for criterion	Data/Basis for scoring	Data source
Food safety	PAH4 level in smoked-dry *Sardinella* sp. (µg/kg)	Minimize impact (PAH4 contamination level in smoked fish)	Actual PAH4 data from the present study	Bomfeh et al., 2019 Chapter 3 (Table 3.1) Chapter 4 (Table. 4.4)
Cost	Upfront investment cost (USD)	Minimize impact	For the FTT and the Chorkor smoker, the reported cost (in USD*) of building each oven was used. The consumer-end product treatment was assigned the same cost as the Chorkor smoker, since its implementation would presuppose the continued use of that oven	Mindjimba, 2019 Ndiaye et al., 2015 Towers, 2014
Exposure to occupational hazards	Amount of carbon monoxide in released smoke during oven use (CO mg/m^3)	Minimize impact	Reported amount of carbon monoxide (CO) released during use of the FTT and Chorkor smoker were used as a proxy for hazardous compound exposure in wood smoke	Anoh et al., 2017
Consumer acceptance of products from the intervention	Sensory evaluation scores for smoked fish from the intervention	Maximize impact (score 3=best case)	Scores assigned based on overall liking score from sensory evaluation. High acceptance = 3, medium acceptance = 2, low acceptance = 1	Chapter 5
Sustained use of intervention	Continued use of intervention following introduction	Maximize impact (score 3=best case)	Reported sustained use after first contact with intervention option. Sustained use = score 3; discontinued use due to dislike of intervention = score 1; discontinued use due to other reasons = score 2	Mindjimba and Tiotsop, 2018
Environmental sustainability	Quantity of fuel used during processing	Maximize impact (score 3=best case)	Comparative fuel consumption. Intervention with least fuelwood consumption = score 3; highest fuelwood consumption = score 1	Anoh et al., 2017 Ndiaye et al., 2015 Olokor, 2003

**USD = United States dollars*

Table 7.2: Criteria scores and weights applied in the scenarios for evaluating interventions for reducing PAH exposure in smoked fish in Ghana

	Criteria scores (actual data or assigned scores on a 3-point scale of 1 = low, 2 = medium, 3 = high)					
Intervention option	**Food safety (PAH4 µg/kg)***	**Cost (USD)**	**Occupational exposure to hazards (CO mg/m^3)****	**Consumer acceptance of products**	**Sustained use of intervention**	**Environmental sustainability**
FTT	2.2	1,668	50	3	2	2
Chorkor smoker	394.5	350	350	3	3	1
Consumer-end treatment	78.2	350	350	2	1	1
Weights for scenarios (%)						
Scenario I***	16.67	16.67	16.67	16.67	16.67	16.67
Scenario II****	30	10	30	10	10	10
Scenario III*****	3.33	30	3.33	30	30	3.33

*Food safety: measured as the mean PAH4 concentration in smoked-dry *Sardinella* sp. processed/prepared with the intervention options

**Occupational exposure to hazards: measured as the reported amount of carbon monoxide (CO) emitted during use of each intervention at the processing step

For the Consumer acceptance, Sustained use of intervention and Environmental sustainability criteria, the higher the score, the more desirable the associated intervention option. For the remaining criteria, the lower the metric value, the more desirable the intervention option

***Scenario I: All criteria considered equally important in the decision making (equal weights assigned)

****Scenario II: Public health prioritized, hence Food safety and Occupational exposure to hazards considered more important than the other criteria

*****Scenario III: Economic viability of intervention prioritized, hence Cost, Consumer acceptance of products and Sustained use of intervention considered more important than other the other criteria

Having scored the criteria, the MCDA outranking software Visual PROMETHEE® (Academic Version 1.4, VP Solutions, Belgium) was used to rank the interventions. The performances were measured as *preference flows* [represented by the Greek letter Phi (Φ)], which is a consolidation of the strengths and weaknesses of each intervention in relation to each evaluation criterion (VP Solutions, 2013). Three Φ values were computed as follows (Brans and Mareschal, 2005):

i. Positive preference flow (Φ+): This is a measurement of the overall strengths of an intervention and indicates how much one intervention is preferred to the others. The larger the value of Φ+, the better the intervention option. For two interventions ***a*** and ***b***, Φ+ is calculated as:

$$\Phi^{+}(a) = \frac{1}{n-1}\sum_{b\neq a}\pi(a,b)$$

where Φ+ shows how much ***a*** is preferred to the other n-1 alternative interventions (in this case, **b**).

ii. Negative preference flow (Φ-): Considering interventions ***a*** and ***b*** as example, Φ- is a measurement of the overall weaknesses of ***a*** and indicates how much ***b*** is preferred to preferred ***a***. The smaller the value of Φ-, the better the corresponding intervention option, and vice versa. Φ- is calculated as:

$$\Phi^{-}(a) = \frac{1}{n-1}\sum_{b\neq a}\pi(b,a)$$

where Φ- shows how much the other n-1 alternative interventions (in this case, ***b***) are preferred to ***a***.

iii. Net preference flow (Φ): This is a balance (i.e. "*net* value") between Φ+ and Φ- It is an aggregation of the strengths and the weaknesses. For example, if Φ+ = 3 and Φ- = 1, then Φ = |3| - |-1| = 2. The larger the Φ, the better the intervention.

The Φ value can be negative or positive. A positive value indicates that the strengths of the intervention outweigh the weaknesses, and vice versa. Note that a negative Φ value is *not* the same as Φ-. Whereas the former is the result of summing Φ+ and Φ-, the latter is an independent value representing weaknesses.

The Visual PROMETHEE® software computes the Φ+, Φ- and Φ and ranks the interventions based on those values. Two types of ranking are performed, namely PROMETHEE I Partial Ranking and PROMETHEE II Complete Ranking (VP Solutions, 2013).

7.1.5.1 PROMETHEE I Partial Ranking

This ranking is based on the Φ+ and Φ - values, where ***a*** is preferred to ***b*** if and only if it is preferred to ***b*** according to both preference flows as follows:

$$aP^{I}b \text{ if and only if } \Phi+(a) \geq \Phi+(b) \text{ and } \Phi-(a) \leq \Phi-(b)$$

where $aP^{I}b$ means "***a*** is preferred to ***b*** in the PROMETHEE I ranking" (VP Solutions, 2013). Thus, in the PROMETHEE I Partial ranking, a preferred intervention must have better Φ+ ***and*** Ph- values than other options.

7.1.5.2. PROMETHEE II Complete Ranking

This ranking is based on the net preference flow (Φ). Therefore, action ***a*** is preferred to action ***b*** in the PROMETHEE II Complete ranking if and only if it has a Φ value greater than the Φ value of ***b*** (VP Solutions, 2013). That is:

$$aP^{II}b \text{ if and only if } \Phi(a) > \Phi(b)$$

where $aP^{II}b$ means "***a*** is preferred to ***b*** in the PROMETHEE II Complete ranking" (VP Solutions, 2013). Thus, in PROMETHEE II Complete ranking, the intervention with the higher net flow (Φ) is ranked higher.

In this study, the outcome of the PROMOTHEE I Partial rankings did not differ from the PROMETHEE II Complete Ranking. Hence, results of the latter are presented. The contribution of each criterion to the strengths, weaknesses and ranks of each intervention were determined by multiplying the individual preference flows (Φ values) of the criteria by their respective assigned weights (VP Solutions, 2013), and the outputs displayed in stacked column charts referred to as PROMETHEE Rainbow charts.

7.2 RESULTS AND DISCUSSION

In this section, the ranking of the interventions based on the Visual PROMETHEE II Complete ranking is presented and discussed. Firstly, the ranking based on a combination of all three scenarios is considered, providing an overview of the performance of each intervention option against all considered weights of the criteria. Secondly, each scenario is considered at a time. For both considerations, results are presented in two formats: a table of preference flow values (i.e. Φ+, Φ- and Φ) and PROMETHEE Rainbow charts. The table indicates the ranks for the interventions and their corresponding Φ+ and Φ- values. The PROMETHEE Rainbow charts then allow a graphical comparison to be made in the same space among *all* the interventions for each scenario, indicating the contribution of the various criteria to the observed rankings.

Visual stability intervals for the various criteria are also discussed. These intervals show the limits of weight adjustments within which the rankings would remain valid and, hence, beyond which they would change (Ruzante et al., 2010).

As explained earlier, although not an intervention, the Chorkor smoker, being the current oven, was also assessed to measure how each intervention option fared against it, and how it also fared on the evaluation criteria.

7.2.1 Intervention rankings across all scenarios

The PROMETHEE preference flow values (i.e. Φ+, Φ- and Φ) for each intervention under the various scenarios are shown in Table 7.3. For each scenario, a positive Φ value indicate that the strengths of an intervention outweigh the weaknesses. The magnitude of Φ then shows how the various interventions compare in their strengths and weaknesses. For example, from Table 7.3, it is seen that across all scenarios, the consumer-end product treatment had negative Φ values, implying that its weaknesses outweigh its strengths. The FTT, on the other hand, had positive Φ values for Scenario I (all weights equal) and Scenario II (public health prioritized), indicating greater strengths than weaknesses in those cases. The magnitude of Φ further highlights differences in strengths or weaknesses. For example, in Scenario II, Φ for consumer-end product treatment (-0.35) was less than that for the Chorkor smoker (-0.30), hence, the former had more weaknesses than the latter.

Across the interventions, the consumer-end product treatment consistently had lower Φ than the FTT. Consequently, in all scenarios, the FTT ranked higher rank (rank 1 in Scenarios I and II, and rank 2 in Scenario III) than the consumer-end product treatment (rank 3 in all scenarios) (Table 7.3). Thus, the FTT performed better than the current situation (Chorkor smoker) and the consumer-end product treatment when all the evaluation criteria were considered equally important as well as when public health was prioritized.

Table 7.3: Overview of PROMETHEE rankings for the two potential interventions for reducing consumer exposure to PAHs in smoked fish in Ghana, compared to the currently-used Chorkor smoker

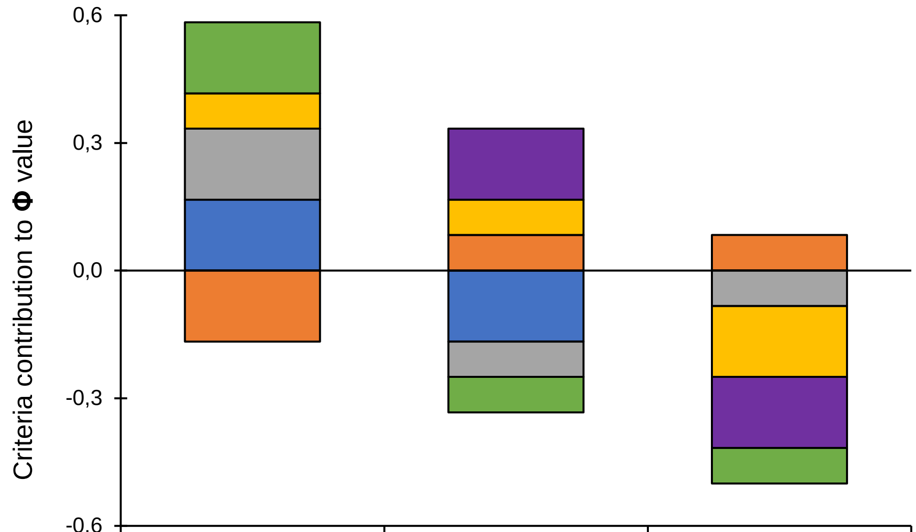
Intervention option	Scenario I: All criteria equally important				Scenario II: Public health prioritized				Scenario III: Economic viability prioritized				All Scenarios*			
	Φ+	**Φ-**	**Φ**	**Rank**	**Φ+**	**Φ-**	**Φ**	**Rank**	**Φ+**	**Φ-**	**Φ**	**Rank**	**Φ+**	**Φ-**	**Φ**	**Rank**
FTT	0.67	0.25	0.42	**1**	0.80	0.15	0.65	**1**	0.40	0.45	-0.05	**2**	0.62	0.28	0.34	**1**
Chorkor smoker	0.33	0.33	0.00	**2**	0.20	0.50	-0.30	**2**	0.60	0.07	0.53	**1**	0.38	0.30	0.080 .07	**2**
Consumer-end product treatment	0.17	0.58	-0.42	**3**	0.20	0.55	-0.35	**3**	0.17	0.65	-0.48	**3**	0.18	0.59	-0.41	**3**

*This is automatically computed by the PROMETHEE® software as an aggregation of the conditions for all the scenarios

7.2.2 Scenario I: All criteria considered equally relevant for decision makers

The PROMETHEE Rainbow chart for Scenario I is shown in Fig. 7.2. In the figure, each column represents an evaluated intervention, arranged from left to right in their rank order (i.e. rank 1 to 3). Portions of the columns above the origin represent strengths (Φ+), whiles weaknesses are shown below the origin (Φ-). The slices within each column show the respective contributions of the criteria to the strengths and weaknesses of each intervention. It is seen that, for strengths, the Φ values decrease from the FTT through to the consumer-end product treatment, and a reversed trend observed for weaknesses. These show that, across the criteria, FTT had more desirable outcomes than the alternatives.

From Fig. 7.2, it is also seen that the weakness of the FTT reported in Table 7.3 for this scenario (i.e. the Φ- value of 0.25) was solely due to a poor performance on the cost criterion, whereas those of the Chorkor smoker and the consumer-end product treatment were due to multiple criteria. The Chorkor smoker was weak in occupational exposure to hazards, food safety and environmental sustainability (Fig. 7.2), whiles the consumer-end product treatment was weak in all criteria except cost. These show that, within this scenario, the FTT was the only option to address the central issue of food safety. However, it is also the only option to show a weakness in the cost criterion. This suggests that it will be effective in addressing the MCDA problem, albeit with a higher financial burden than the alternative and the status quo.



	FTT	Chorkor smoker	Consumer-end action
Environmental sustainability	0,2	-0,08	-0,08
Sustained use	0,00	0,2	-0,2
Consumer acceptance	0,08	0,08	-0,2
Occupational hazard exposure	0,2	-0,08	-0,08
Cost	-0,2	0,08	0,08
Food Safety	0,2	-0,2	0

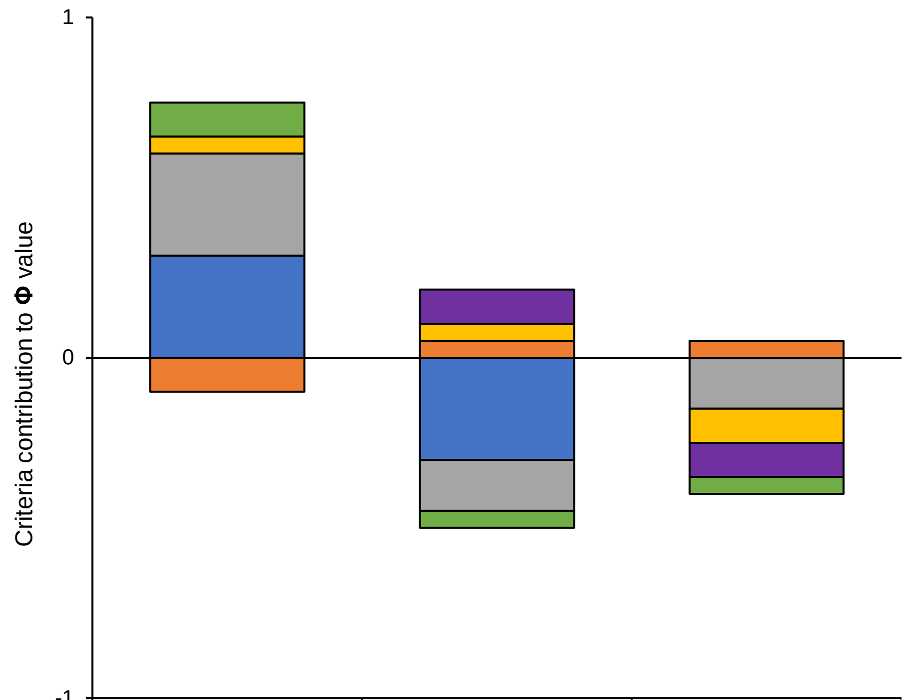
Fig. 7.2: PROMETHEE Rainbow chart for Scenario I: All six criterial considered equally relevant for decision makers, expressing the contributions of decision criteria to the overall strengths and weaknesses of three intervention options (horizontal axis) for reducing human exposure to PAHs in smoked fish in Ghana. Positive values (slices above the origin) denote strengths; negative values (slices below the origin) denote weaknesses.

It is significant to note that the cost criterion was defined within the limited scope of the required upfront investment for each intervention. If this cost of implementation is combined with other indirect costs – for example, costs for medical care due to morbidities arising from occupational exposure to processing hazards – the ranking dynamics on cost may change in favour of the FTT, since the FTT reduces such exposure. Moreover, in the case of considering the economic impact of morbidities, the added pressure on the national healthcare system (and hence national budgets) may also be considered in the cost element, further deepening the potential gap in the ex-ante ranking. Although these two costs are different (cost of implementation and cost of illness), they reflect the same practical reality - economic burden linked to the intervention options.

From Fig. 7.2 and Table 7.3, it is also seen that the strengths of the FTT were due mainly to good performances on food safety, occupational exposure to hazards and environmental sustainability, each accounting for 25% of the Φ+ for the FTT. The key strength of the Chorkor smoker was the sustained use criterion, invariably due to its long-standing history of use (since 1969, see Chapter 1).

7.2.3 Scenario II: Public health prioritized (Food safety and occupational hazard exposure considered more important than the other criteria)

When public health was prioritized, the ranking did not differ from that in Scenario I (i.e. FTT = 1; Chorkor smoker =2; Consumer-end product treatment = 3) (Fig. 7.3). However, the contributions of the various criteria to the strengths and weaknesses of the interventions were altered in some cases. For example, in Scenario II, food safety accounted for 38% of the overall strengths (Φ+) of the FTT (cf. Fig. 7.3 and Table 7.3), whereas its share in Scenario I was 25% (cf. Fig. 7.2 and Table 7.3). For the Chorkor smoker, the contribution of food safety to its overall weaknesses was 60%, whereas the value in Scenario I was 50%. Similarly, the contribution of occupational hazard exposure to the strengths of the FTT increased from 25% in Scenario I to 38% in Scenario II, whereas the contribution of the same criterion to the weaknesses of the Chorkor smoker increased from 25% in Scenario I to 30% in Scenario II. For the consumer-end treatment, its performance on food safety was neutral (neither weak nor strong), suggesting that the concern will not be addressed with this intervention. Occupational hazard exposure however accounted for 27% of its weaknesses in Scenario II, up from 14% in Scenario I. These show that when public health is made the central issue for the decision-making, the suitability of the FTT as an intervention for human exposure to PAHs in smoked fish in Ghana becomes even more pronounced, and the inadequacy of the consumer-end product treatment and the status quo (Chorkor smoker) correspondingly evident.

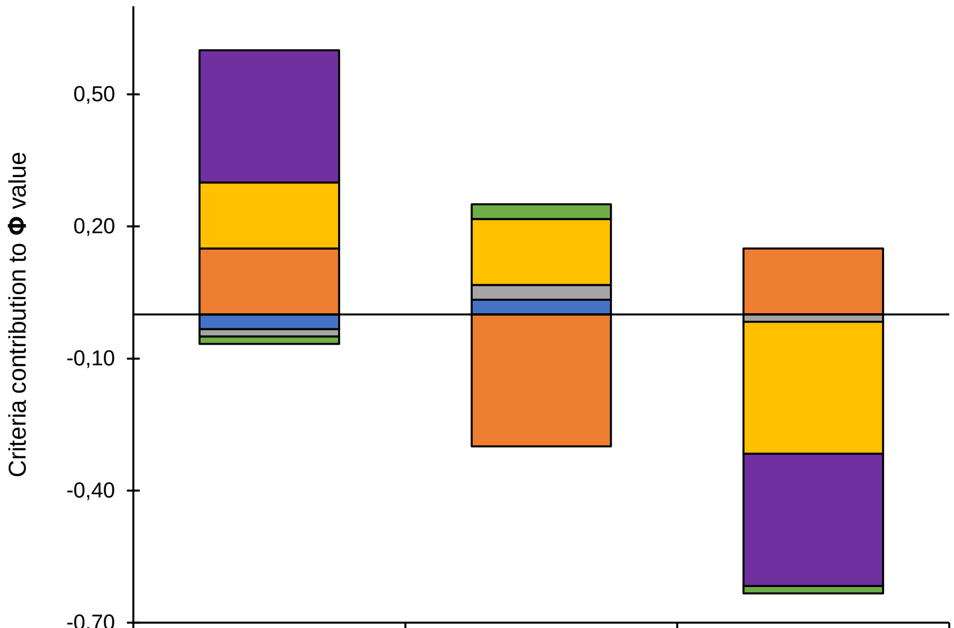


	FTT	Chorkor smoker	Consumer-end action
Environmental sustainability	0,1	-0,05	-0,05
Sustained use	0	0,1	-0,1
Consumer acceptance	0,05	0,05	-0,1
Occupational hazard exposure	0,3	-0,15	-0,15
Cost	-0,1	0,05	0,05
Food Safety	0,3	-0,3	0

Fig. 7.3: PROMETHEE Rainbow chart for Scenario II: Public health prioritized, expressing the contributions of decision criteria to the overall strengths and weaknesses of three intervention options (horizontal axis) for reducing human exposure to PAHs in smoked fish in Ghana. Positive values (slices above the origin) denote strengths; negative values (slices below the origin) denote weaknesses.

7.2.4 Scenario III: Economic viability of intervention prioritized (Cost, consumer acceptance of products and sustainable use of intervention considered more important)

In this scenario, the Chorkor smoker ranked higher (rank 1) than the FTT (rank 2), suggesting that when implementation cost, potential sustained use and consumer response to products are considered most important by the decision maker, the status quo (Chorkor smoker) outperforms the two interventions. However, consumer exposure to PAHs remains compromised (Fig. 7.4).



	Chorkor smoker	FTT	Consumer-end action
Environmental sustainability	-0,02	0,03	-0,02
Sustained use	0,3	0	-0,3
Consumer acceptance	0,2	0,2	-0,3
Occupational hazard exposure	-0,02	0,03	-0,02
Cost	0,2	-0,3	0,15
Food Safety	-0,03	0,03	0

Fig. 7.4: PROMETHEE Rainbow chart for Scenario III: Economic viability of intervention prioritized (Cost, consumer acceptance of products and sustainable use of intervention considered more important), expressing the contributions of decision criteria to the overall strengths and weaknesses of three intervention options (horizontal axis) for reducing human exposure to PAHs in smoked fish in Ghana. Positive values (slices above the origin) denote strengths; negative values (slices below the origin) denote weaknesses.

This points to the need for stakeholder engagement, especially among risk managers on the one hand and food business operators (FBOs, particularly fish processors) and consumers on the other hand, regarding the harmful effects of the continued use of the traditional ovens and how the FTT could address such concern. With appropriate support, the economic viability of the FTT could be realized not only domestically, but also on the export market. For example, between 2006 and 2011, Cote d'Ivoire lost one billion CFA (about two million USD) due to a self-ban on smoked fish exports on account of high PAH alerts from the EU. When the FTT was introduced in that country, exporters who adopted the innovation were able to resume business (Peñarubia et al., 2017). There is, therefore, an economic sustainability case to be made for the FTT. However, appropriate stakeholder engagement and support are needed to prepare the domestic market for the products, and thus incentivise processors to adopt and continue to use the oven.

7.2.5 Stability intervals analysis

The stability intervals for the criteria are shown in Table 7.4. The wider the range, the less constrained is the choice for weights, and vice versa (Ruzante et al., 2010). For example, for the environmental sustainability criterion, an interval of 0 – 100% implies that any weight can be selected within that range and applied to that criterion without causing a change in the rankings for the specific scenario considered. For stability intervals with an upper limit less than 100%, any weight exceeding that limit would result in a change in the ranking obtained with the original weights. For example, from Table 7.4, the cost criterion for Scenario I has a stability interval of 0 – 35%. In that scenario, the originally assigned weights were 17% per criterion, and the ranking order was FTT 1, Chorkor smoker 2, and consumer-end product treatment 3. Therefore, increasing the assigned weight of cost beyond 35% would change the ranking order, for that scenario, as shown in Table 7.5. The table thus highlights how the rankings would change should stakeholders assign weights different from those used in the study, while maintaining the scores/values for the various metrics.

Table 7.4: PROMETHEE Stability intervals for the rankings of the three scenarios for reducing consumer exposure to PAHs in smoked fish in Ghana (Percentages rounded up to the nearest whole number)

	Scenario I		Scenario II		Scenario III	
Criterion	**Weight (%)**	**Interval (%)**	**Weight (%)**	**Interval (%)**	**Weight (%)**	**Interval (%)**
Food safety	17	0 – 41	30	0 – 31	3	0 – 25
Cost	17	0 – 35	10	0 – 45	30	0 – 46
Exposure to occupational hazards	17	0 – 100	30	0 – 100	3	0 – 34
Consumer acceptance of products from intervention	17	0 – 100	10	7 – 100	30	2 – 100
Sustained use of intervention	17	0 – 41	10	7.2 – 54	30	0 – 100
Environmental sustainability	17	0 – 100	13	0 – 100	3	0 – 30

Table 7.5: Changes in intervention ranks due to exceedance of stability intervals of the criteria for the three evaluated scenarios

	Scenario I				Scenario II				Scenario III				
Rank	**Original ranking**	**Weight of *Food Safety* increased to 42%**	**Weight of *Cost* increased to 36%**	**Weight of *Sustained use of intervention* increased to 42%**	**Original ranking**	**Weight of *Food Safety* increased to 32%**	**Weight of *Cost* increased to 46%**	**Weight of *Sustained use of intervention* increased to 55%**	**Original ranking**	**Weight of *Food Safety* increased to 26%**	**Weight *Cost* increased to 47%**	**Weight of *Occupational hazard exposure* increased to 35%**	**Weight of *Environmental sustainability* increased to 31%**
1	FTT	FTT	Chorkor smoker	Chorkor smoker	FTT	FTT	Chorkor smoker	Chorkor smoker	Chorkor smoker	FTT	Chorkor smoker	FTT	FTT
2	Chorkor smoker	Consumer treatment	FTT	FTT	Chorkor smoker	Consumer treatment	Consumer treatment	FTT	FTT	Chorkor smoker	Consumer treatment	Chorkor smoker	Chorkor smoker
3	Consumer treatment	Chorkor smoker	Consumer treatment	Consumer treatment	Consumer treatment	Chorkor smoker	FTT	Consumer treatment	Consumer treatment	Consumer treatment	FTT	Consumer treatment	Consumer treatment

It must be underscored that making decisions on these adjusted rankings outside the context of the decision goal may lead to misleading conclusions. In this study, the goal was to reduce consumer exposure to PAHs in smoked fish. Thus, any weight adjustments that diminish the potential to realize this goal should generally be avoided. In all cases where the original rankings were altered due to weight changes (Table 7.5), food safety remained compromised for the Chorkor smoker and the consumer-end product treatment, occurring as a weakness for those options, but as a strength for the FTT. Thus, within the context of the decision goal, the higher rankings for the Chorkor smoker following the weight adjustments (Table 7.5) do not translate into real benefits. Furthermore, the outputs of the PROMETHEE software should be seen to aid – not replace – the critical evaluation of intervention options by decision makers (Ruzante al., 2010; FAO, 2017).

7.3 CONCLUSION

Within the context of the evaluation conditions presented, the PROMETHEE MCDA evaluations ranks the FTT as a better intervention than the consumer-end product treatment for reducing PAH exposure in smoked fish in Ghana. The Chorkor smoker (the status quo) was also found to perform better than the consumer-end product treatment, further underscoring the unreliability of the latter as an intervention for reducing human exposure to PAHs in smoked fish. Prioritizing economic viability – defined in the restricted sense of upfront investment, sustained use of, and consumer acceptance of products from an intervention – results in the Chorkor smoker ranking higher than the FTT. However, the declared goal of the decision-making endeavour is not met in that case, since the Chorkor smoker retains its weaknesses in public health protection. An important drawback of the presented MCDA is the lack of actual stakeholder consultation for the identification of decision factors and subsequent assignment of weights.

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In the next and final chapter, the findings of all the studies are discussed in relation to the topic of the thesis, conclusions drawn, recommendations made for context-relevant implementation of research findings, and suggestions offered for future research.

CHAPTER 8: GENERAL DISCUSSION, CONCLUSIONS AND FUTURE PERSPECTIVES

8.1 Overview

In this final chapter, the results of the whole study are discussed, the key findings highlighted and overall conclusions and recommendations provided for future research. The discussions are presented within the broader context of the study objectives and against the background of the food security significance of smoked fish in Ghana, the important role of artisanal food processors (majority of them women) in ensuring availability of fish in preserved form all-year-round, the food safety problems associated with the unsophisticated approaches used to process and store fish, and the food safety governance challenges that arise from managing health risks in a context with limited resources.

8.2 Safety of traditionally smoked fish in Ghana

In **Chapter 2**, it was shown that, for food safety governance of smoked fish in Ghana, PAHs were more relevant than microbial hazards (represented by *Salmonella* sp.) and biogenic amines (represented by histamine). This does not suggest that the other hazards are not important. The Foodborne Disease Burden Epidemiology Reference Group (FERG) of the World Health Organization (WHO), for example, reports that microbial hazards caused the most foodborne illnesses globally (WHO, 2015). Thus, the results of the chapter show where urgent action is needed as far as traditionally smoked fish in Ghana is concerned. Basic good manufacturing practices (GMP), good hygienic practices (GHP), and hygienic handling of food at the consumer level should be continuously encouraged to keep the other hazards in check.

As earlier noted, Ingenbleek et al. (2019) also reported that smoked fish was the important food item linked to excessive PAH contamination in sub-Saharan Africa. The findings reported in **Chapter 2** show that, at the national level, this is the case in Ghana, and thus calls for an intervention in that local context.

Another issue highlighted in **Chapter 2** is the role of post-processing contamination on the quality and safety of smoked fish. The higher microbial loads in the products from the markets point to unsatisfactory hygienic conditions. Products on the markets are displayed in the open, under ambient tropical temperature and without packaging, thus exposing them to microbial contamination and proliferation (Fig. 8.1). Improvements in the layout of the markets, provision of appropriate stalls and context-relevant product packaging options may be useful.

For the PAHs, the higher levels in the market products were ascribed to the practice of re-smoking. The fish are re-smoked to prevent or eliminate insect infestation (Ahadzi, 2017). Since the problem of insect infestation invariably results from the lack of adequate packaging and storage facilities, should supporting infrastructura be provided, the practice may not be needed.

It is acknowledeged that the sample size (80 total) was rather small for an assessment of a national situation. A larger sample size, covering a wider perimeter than was considered in the study may have enhanced the generalizability of the study findings. Also, only hazards potentially linked to processing and handling were considered. These, therefore, should not be construed to be the only hazards associated with smoked fish. For example, raw material quality is an important determinant of food product quality. Hence, for a comprehensive assessmsent of important hazards in smoked fish, more hazards need to be considered beyond those included in this study, covering all stages of the smoked fish value chain.

Fig. 8.1: Smoked fish for sale on a market in Ghana[5] showing simple infrastructure that do not support hygienic handling

[5] Source: https://www.dw.com/en/raising-money-from-catfish-in-ghana/a-44406376

The hazard scoring proof of concept presented in **Chapter 2** could find application in situations where hazard concentration and prevalence data are available. As was illustrated, its semi-quantitative nature allows a more nuanced categorization of hazards and captures food safety concerns that may be missed when decision flowcharts are used. It is not, however, proposed as an outright better alternative to flowcharts. The choice of screening method shoud be informed by the available data on the ground.

As with any hazard screening method, the use of the hazard scoring should be considered as a first step to a risk assessment or a risk ranking. It should be emphasized, however, that the validity of the hazard scoring output depends on the quality and reliability of the available data. Poor sampling and analytical procedures will lead to unreliable conclusions about the generalizability of the hazard screening.

- **Key Finding 1**: PAHs are the most relevant food safety hazards in smoked fish in Ghana, as far as the processing technique and product handling are concerned

- **Key Finding 2**: Post-processing contamination is an important contributor to the poor microbial quality of smoked fish on markets (potentially due to unsatisfactory hygiene at the market and improper handling and packaging). Re-smoking of unsold/stored smoked fish increases the already high PAH levels in the product

- **Key Finding 3**: The presented hazard scoring proof of concept may be used in screening hazards in particular contexts when hazard prevalence and concentration data are available. The validity of its outputs, however, will depend on data quality.

8.3 Efficacy of the FTT to yield smoked fish with low and acceptable PAH levels

In **Chapter 3**, it was shown that products from the FTT had lower PAH levels than current EU limits (up to ten-times lower than the BaP limit of 2 µg/kg, and up to eight times lower than the PAH4 limit of 12 µg/kg; Commission Regulation (EC) No. 835/2011)). In contrast, products from the Chorkor smoker exceeded the limits by up to 31-fold for BaP, and up to 33-fold for PAH4. For the metal drum, the exceedance was up to 19-times for BaP, and up to 27-times for PAH4. These figures highlight on the one hand, that use of the traditional ovens results in elevated contamination of PAHs in smoked fish, and on the other hand, that the FTT is technically viable for addressing the problem. In general, the differences were attributed to differences in oven design and type of fuel used.

Concerning oven design, it is noted that the features of the FTT were engineered to leverage on the provisions in Codex normative instruments for reducing the extent of PAH contamination during food smoking (CAC/RP 68 – 2009; CODEX STAN 311 – 2013). The separation of the cooking and smoke flavouring unit operations, the fat collector appendage, the indirect smoke generation and filtering are all design characteristics that contributed to the lower PAH levels in the FTT products (Ndiaye et al., 2015).

For the impact of fuel type, the observation that use of fuelwood (instead of charcoal) on the FTT caused violation of the EU limits for PAHs whiles use of charcoal (instead of fuelwood) on the Chorkor smoker resulted in lower PAH levels that still exceeded the EU limits, highlights the important contribution of fuelwood to PAH contamination of smoked fish. It also shows that, merely changing the type of fuel used on the Chorkor smoker is insufficient to obtain satisfactory PAH contamination levels in products smoked fish. Since the metal drum oven yielded products with lower PAHs than the Chorkor smoker, it is probable that if charcoal is used as fuel on that oven (i.e. metal drum), the PAHs would be further reduced. However, whether such reduction would satisfy regulatory limits needs to be studied. Among other things, it may serve to strengthen the evidence for the assertion that both oven design and fuel type are important for controlling PAH contamination in smoked fish.

8.3.1 Impact of FTT parts on PAH contamination of smoked fish

Concerning the observation that when the fat collector was not used, PAH levels in FTT products exceeded the EU limits (2.9±0.2 µg/kg in product vs 2.0 µg/kg limit for BaP; 39 ± 2µg/kg product vs. 1 2µg/kg limit for PAH4), it should be noted that the quantities of fish smoked per session in the study (18kg) is much lower than would be processed for commercial purposes. Thus, the quantity of fat exudates, and hence the corresponding pyrolytic production of PAHs (Šimko, 2002) would be much higher when commercial quantities of fish are smoked on the oven. This underscores the need to keep the fat collector in place when using the FTT.

For the type of material for generating smoke to flavour fish (sugarcane bagasse, coconut husk and *Azadirachta indica* wood), although statistically significant differences were reported for the PAH levels, the practical relevance of the differences is minimal since none of the materials caused violation of the EU limits. However, in support of environmental sustainability, it may be more appropriate to use either the sugarcane bagasse (fibrous waste from extraction of sugarcane juice) or the coconut husk (agriwaste from coconut). Similarly, the results also suggest that the indirect smoke generator and filtering unit are not essential for reducing PAH contamination. When the filter was not used, PAH levels in smoked products were well below the regulatory limits (e.g. 0.9 µg/kg BaP vs. regulatory limit of 2 µg/kg; and 8 µg/kg PAH4 vs. regulatory limit of 12 µg/kg). The unit could thus be considered dispensable. This will reduce the cost of constructing the oven and potentially boost its adoption.

- **Key finding 4**: The FTT is effective in yielding smoked products with PAH levels that are lower than current EU limits (Commission Regulation (EC) No. 835/2011)

- **Key finding 5**: Oven design and fuel type are both important determinants of PAH contamination during fish smoking. On either aspect, the FTT shows greater strength than the Chorkor smoker and the metal drum oven

- **Key finding 6**: Using fuelwood instead of charcoal on the FTT results in violation of the EU limits for PAHs. Not using the fat collector also results in violation of the limits.

- **Key finding 7**: The indirect smoke generator of the FTT is dispensable, as the PAH levels in products of the oven fall below regulatory limits whether the filter was used or not.

8.4 Risk assessment of PAHs in smoked fish in Ghana

In **Chapter 4**, it was shown that consumer exposure to PAHs in smoked fish from the Chorkor smoker and the metal drum oven were high (MoE <10,000) and that exposure in FTT products was low (MoE >10,000), illustrating the technical suitability of the latter as an intervention for the high exposure. It was also shown that although consumer-level product treatment reduced PAH contamination in smoked fish, it did not reduce overall exposure to the hazards. Although the risk assessment provides some insights into potential PAH public health risks of traditionally smoked fish consumption, its outputs coud be further improved. For example, an average adult body weight of 60kg was used. Had a distribution been used instead of the single value, weight variabilities in the population may have been captured to improve the risk estimate. Also, in Scenario III (removal of the skin of smoked fish) the same consumption pattern for the unskinned product was assumed. In reality, however, since this practice is not common, the consumption pattern is expected to be different (lower intakes than unskinned fish), with a consequent potentially lower exposure to PAHs in those products. Furthermore, only one species of fish was considered. Variabilities in preferences for other species of fish would affect overall exposure to PAHs in smoked fish in general.

The high consumer exposure in the traditionally smoked products should provide the impetus for the competent authorities in food safety in Ghana to develop and enforce appropriate, evidence-based regulations for the hazard in smoked products, to protect the domestic consumer. As noted in Chapter 1, the development and introduction of the FTT were, in fact, informed by quality demands of the export market (Peñarubia et al., 2017). Efforts should be made to protect the domestic consumer as well, without minimizing attention on meeting export requirements (Unnevehr, 2014). Good domestic public health status and good revenue inflow from foreign exchange are both needed for sustainable development in LMICs.

Developing and enforcing food safety regulations in LMICs would be justified if a corresponding (technologically) enabling environment is provided to food business operators. It would be unrealistic to expect compliance to regulations on PAH contamination when the use of the same smoking techniques causing the elevated hazard exposure is not discontinued. Since the FTT reduces consumer exposure, its use could satisfy the improved oven requirement, as part of an integrated approach for addressing the problem.

The Fisheries Commission of the Ghana Ministry of Fisheries and Aquaculture Development (MoFAD) has been working with the Ghana Standards Authority and the SNV[6] Netherlands Development Organization to develop a voluntary three-tier classification system for smoked fish quality in Ghana, with a view to encouraging processors to adopt measures for improving smoked fish quality and safety (Owusu, 2019). In the system, Class 3 (the highest quality class) is to be assigned to products of export quality (implying use of an improved oven); Class 2 (middle quality class) to be assigned to products made by processors applying good manufacturing practices (GMP) and good hygienic practices (GHP) in preparation for hazard analysis critical control point system (HACCP) implementation, and Class 1 (entry level class) to be assigned to products of processors who register on the scheme for initial training. Some challenges can be foreseen in the implementation of the classification scheme, such as development of appropriate packaging for the products (to make labelling possible), provision of appropriate infrastructure to support marketing of the products and prevention of fraud by non-certified processors. These notwithstanding, the step is a laudable move towards a participatory approach to addressing the food safety problem. It will be important, however, to clearly include the reduction of PAH levels in products as an important objective, to protect the domestic consumer.

- **Key Finding 8**: Consumer exposure to PAHs in smoked fish in Ghana is high (MoE <10,000) and thus requires risk management action to protect public health. The high exposure is due principally to the use of traditional ovens for fish smoking.

[6] Stichting Nederlandse Vrijwilligers ("Foundation of Netherlands Volunteers")

Therefore, an oven intervention may be useful for addressing the problem.

- **Key Finding 9**: The FTT is effective in reducing consumer exposure to PAHs in smoked fish to acceptable levels (MoE>10,000), whereas consumer-level product treatment (removing the skin of smoked fish) does not reduce overal exposure.

8.5 Consumer acceptance of FTT products

As presented in **Chapter 5**, results of the sensory evelaution suggests that in Ghana, consumer acceptance of FTT products may not be a challenge, since the overall liking scores for the FTT product did not differ from the scores for the Chorkor smoker product. However, since the majority (64%) of assessors were aged between 18 to 24 years, uncertainties remain concerning the response of older age groups. Additionally, only one species of fish (chub mackerel) and one product type (smoked-soft) were tested, hence there are uncertainties about consumer response to other fish species and product types. In light of these knowledge gaps, the generalizability of the results to the overall population of Ghana and other smoked fish products may be limited.

It is also probable that if consumers are educated about the harmful effects of PAH exposure, the levels of the hazard in smoked fish in Ghana, the contribution of the use of traditional ovens to such hazard levels and the efficacy of the FTT in reducing same, the acceptance of the FTT products would increase further (Mindjimba et al., 2019).

- **Key Finding 10**: Consumer acceptance of smoked chub mackerel from the FTT did not differ from that of the Chorkor smoker (among a consumer base largely composed of young (18 to 24 years) respondents).

8.6 Oxidative stability of lipids in smoked fish as influenced by smoking oven type and duration of traditional storage

In **Chapter 6**, an exploratory study of the oxidative stability of lipids in stored smoked fish was presented, highlighting that lipids in the FTT product were less stable compared to products of the Chorkor smoker and metal drum oven. This may negatively influence consumer acceptance of traditionally stored FTT products, since lipid oxidation is known to produce off-flavours (Schaich, 2005), that may thus compromise the sensory appeal of the (stored) products. The safety of the FTT products may also be compromised due to the presence of the oxidation products (Schaich, 2005; Frankel, 2012), as will the nutritional value vis-à-vis the reduction of omega-3 fatty acid levels through oxidation (Cropotova et al., 2019).

This points to the need to address lipid oxidation such that the solution of one problem (lowering PAH contamination by the use of the FTT) does not lead to the creation of another (increasing lipid oxidation in smoked fish by the use of the FTT). It should be noted that the lipid stability assessment was done considering only traditional storage conditions, since other methods such as cold storage were not available, and will not, at present, be economically accessible to processors. For consumers, although refrigerated storage may be feasible for some, it will not reverse oxidation that has already occurred from traditional storage at the processor-end. In this respect, the feasibility of packaging and cold storage infrastructural support for fish processors should be considered by decision makers. It is expected that challenges will arise with such efforts (e.g. erratic power supply affecting sustainable use of cold storage facilities, increased cost of packaged smoked fish and consumer response to same), but so could opportunities be found to address each challenge as potential business ventures by the private sector.

Concerning the semi-quantitively determined amounts of EPA- and DHA-derived epoxides in the smoked fish fat extracts and the deliberately epoxidized decapsulated fish oils, the findings suggest that these PUFAs are less susceptible to epoxidation than others and may be poor indicators of lipid oxidation in (smoked) fish oils. The occurrence of epoxides and their food safety significance may, however, be interesting to look into especially considering their high levels in apparently minimally oxidized vegetable oils (Mubiru et al., 2013) and the lack of research data (beside this exploratory work) on the occurrence of such oxidized species in fish lipids.

- **Key Finding 11**: Lipids in traditionally stored FTT smoked fish are less stable than in traditionally stored Chorkor smoker and metal drum oven products. This may compromise consumer acceptance of the stored products (due to potential off-flavour development), omega-3 fatty acid contents and the safety of the products vis-à-vis lipid oxidation products

- **Key Finding 12**: The occurrence of derived epoxides of EPA and DHA in the extracted fat of smoked fish was low, limiting the potential use of those specific oxidized species as indicators of lipid oxidation in smoked fish lipids. However, derived epoxides of other unsaturated fatty acids may be present in appreciable amounts and thus invites an investigation into the potential use of total epoxide content as an indicator of oxidation in fish lipids.

8.7 Multicriteria decision analysis for selecting an intervention to reduce consumer exposure to PAHs in smoked fish in Ghana

In **Chapter 7**, a case for multicriteria decision analysis (MCDA) for selecting an intervention for reducing consumer exposure to PAHs in smoked fish was presented. It was shown that, within the limits of the decision factors considered, the FTT ranked as the preferred intervention. It must be emphasized that the presented MCDA analysis was based on selection of factors and weight distribution determined by the author from knowledge and experience of the context. These, therefore, need to be further validated by stakeholder consultation in the country for a national policy decision on the adoption of an intervention.

For a stakeholder-informed MCDA, representatives in government agencies and ministries for the fisheries value chain, fisherfolk and fish processors and traders associations, regulatory authorities, researchers and development partners could be involved in (focus group) discussions where intervention options and factors that ought to be considered in assessing their operationalizability could be solicited, debated, refined and finalized by consensus.

Each group of stakeholders would then assign weights to the factors based on their evaluation of the relevance of such factors to the solution in general, and to their interests in particular. Such a participatory, transparent approach for gathering data for the MCDA would lead to a compromise decision that may be fairly representative of the actual needs and voices on the ground.

The terms used should also be transparently defined in the context. For example, in **Chapter 7**, the “Cost” criterion was defined in the oversimplified form as the initial investment required for an intervention option. In a more thorough evaluation, other elements such as operational and maintenance costs should also be considered and measured against the potential returns on investment. For example, it is estimated that three tonnes of fresh fish is required to meet the maximum daily capacity of the FTT (Zelasney et al., 2020), suggesting that consistently processing volumes below this threshold may not be cost-effective.

Further on costs, in the present MCDA, it was not specified who bears the financial burden for the FTT intervention option (whether food processors or government), although it is expected that for a sustainable business, fish processors would bear the cost of the oven installation, while government bears the cost of consumer awareness campaigns. These, however, should be clearly defined and evaluated in a full-blown MCDA.

Concerning environmental sustainability, it was noted that the FTT has been reported to emit less greenhouse gasses during use than the Chorkor smoker (Ndiaye et al., 2015; Anoh et al., 2017) due to use of charcoal instead of fuelwood as fuel. However, during charcoal production, greenhouse gasses are emitted and should also be factored into assessing the overall impact of each oven on climate health.

On the sustainability of intervention use (i.e. continued long-term use of the FTT following introduction), it was indicated that in some countries in sub-Saharan Africa, use of the oven was discontinued due challenges such as non-price differentiation of the products on the domestic market (Mindjimba and Tiotsop, 2018). In the countries surveyed by

Mindjmba and Tiotsop (2018) (e.g. Cameroon, Côte d'Ivoire and Tanzania), the FTT ovens were built in designated locations for communal use, to reduce the initial investment burden on processors. However, communal use necessarily requires commute for processing. This contributed to the abandonment of the oven over time (ibid.), due to the inconvenience of carting fish between homes and processing centres, among other reasons. Since fish smoking in Ghana (and Africa in general) has historically been a cottage business, processors are used to working and keeping the home in the same space. Commuting also comes with added costs that may not necessarily reflect in the final selling price of the smoked fish.

Another factor identified by Mindjimba and Tiotsop (2018) for the abandonment was the number of ovens per processing centre. Due to the limited number of installed FTT units, communal use was necessary. Communal oven use also requires a schedule for fair allotment of processing time to the processors. This may not always be convenient since processors may prefer the flexibility of processing whenever they have fresh fish (Mindjimba and Tiotsop, 2018). It is probable, therefore, that in non-communal use of the ovens (i.e. individually owned ovens), long-term use may be achieved. This, however, requires significant upfront investment from the processors, and points to the need for establishing appropriate financially enabling arrangements (such as co-operative financing schemes and financial incentives from government) for the processors.

Randrianantoandro and Diei-Ouadi, (2015) surveyed women fish processors in Benin, Côte d'Ivoire, Ghana, Kenya, Togo, Uganda and Tanzania and reported that the processors appreciated the comparative advantages of the FTT and recommended its use. This shows that the willingness to use the innovation is present. However, support for sustainable use should be provided, within the context of local needs and preferences.

In Ghana (and other LMICs), therefore, conducting a national MCDA on interventions for PAH exposure should elicit all the necessary contextual factors through appropriate stakeholder engagement to arrive at a relevant, workable decision.

- **Key Finding 13**: Within the limits of the MCDA decision objective, factors and weights considered in this study, the FTT was ranked as the preferred intervention for reducing consumer exposure to PAHs in smoked fish in Ghana. It however requires a higher upfront investment.

8.8 Risk communication on PAHs in smoked fish in Ghana

As noted in Chapter 1, the aim of risk communication is to promote understanding and dialogue among stakeholders about decisions concerning the management of food safety risks, and to help consumers make informed judgements about food safety hazards and risks (EFSA, 2012). Openness and transparency about the nature or risks and how these influence(d) risk management options are important pillars of risk communication (EFSA, 2012). Therefore, concerning the elevated PAH contamination in smoked fish linked to the use of traditional ovens, and the demonstrated efficacy of the FTT to reduce such communication, it is important to make all stakeholders, especially processors and consumers, aware of the situation. However, in doing so, a fine balance must be struck between highlighting the risks associated with the traditional ovens and highliting the potential benefits of the FTT.

For example, while consumer education on the benefits of the FTT would ultimately be geared towards garnering public acceptance of the products of the oven, which would in turn engender the buy-in of processors for the adoption of the oven, it is crucial to ensure that such education is delivered in a language that does not undermine the livelihoods of the processors (Mindjimba et al., 2019). In Ghana, when evidence from this study was shared in a dissemination workshop (see Section 8.9), some media reportage on the matter inflamed passions rather than constructively informing the public (see Fig. 8.2) (Ofori, 2017). Whereas some consumers panicked at reports on the risks associated with traditionally smoked fish, others downplayed the news, citing the long history of consuming smoked fish from the traditional ovens without (obvious) health problems. The processors, on the other hand, were concerned about the impact of reports on their business.

Fig. 8.2: Media report in Ghana (9 September, 2017) on an event organized to share the results of this study on the efficacy of the FTT. Needless panic was caused among processors and consumers due to the wording of the report.

Similarly, in Nigeria, media coverage of an event introducing the FTT also used language that triggered panic among consumers and processors (Fig. 8.3) (Metrowatch, 2015). Messages delivered in such manner may erode consumer confidence in domestically produced smoked fish, regardless of the type of oven used for processing. Furthermore, it may weaken processor willingness to adopt the FTT. Consumer education and stakeholder engagement as part of risk communication should therefore use language that communicates the benefits of the FTT without demonizing the traditional ovens and their users (Mindjimba et al., 2019).

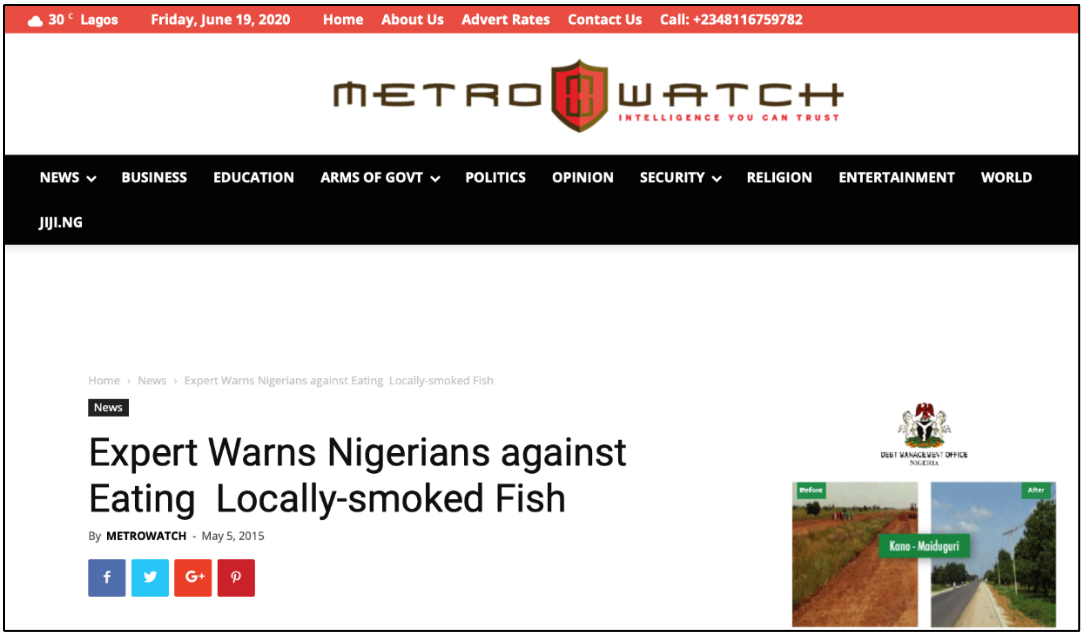


Fig. 8.3: Media report in Nigeria (5 May, 2015) on an event organized to share the benefits of the FTT oven over traditional smoking ovens. The reporters sensationalized the matter by focusing on the problem associated with the traditional ovens

A positive example of such communication is shown in Fig. 8.4, in which a media report in Sri Lanka emphasized the health and economic benefits of using the FTT, without unnecessarily downgrading the traditional ovens.

Innovative ovens for fish-smoking improve Batti lives

The traditional method of fish-smoking in Unnichchai, a small fishing community in the Batticaloa district sees women spending many hours tending to fish laid out on mesh over smoking coals. Health hazards from smoke inhalation are high, while the output from such intensive labour is often low. That is now changing with the introduction of new smoking ovens pioneered by the Food and Agriculture Organisation of the United Nations (FAO) under a programme funded by the European Union (EU), a joint media release said.

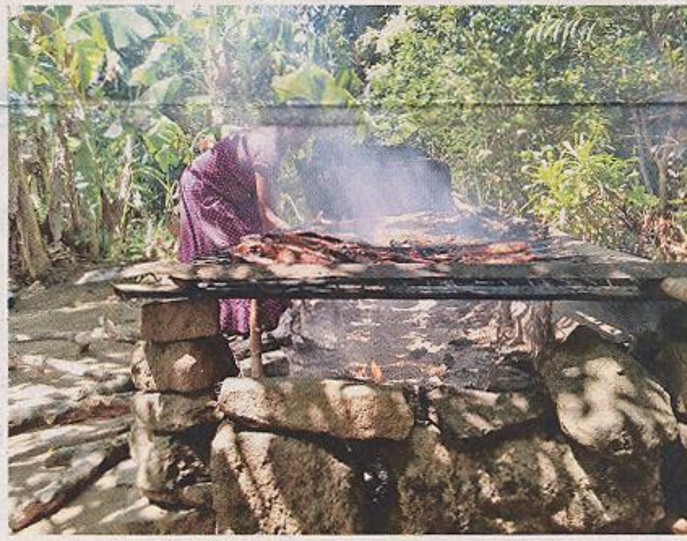
"The first thing I noticed was that the new technology helps me save time," said Kopalapillai Theivarmallar who makes her living from fish-smoking to provide for her three daughters. "Before, I spent a total of 12 hours on two consecutive days drying and smoking the fish, and I used the old method of iron mesh. Now, 6 to 7 hours are enough to finish one smoking. It gives me plenty of time to take care of my children and do household chores," she said.

Since 2008 FAO has worked on developing the FTT-Thiaroye, an improved fish smoking and drying oven technology. The oven can be purpose built, or the smoke-capturing chimney, oil-catching trays and other elements can be added to an existing oven. It is designed to improve fuel-efficiency in fish-smoking by encapsulating heat and smoke. It also addresses health hazards suffered by small-scale fish dryers - the vast majority of whom are women.

In the Batticaloa district, around 150 families are engaged in fish-smoking activities. The traditional smoking method is done in the open, making the fish prone to spoilage from rain and external contamination. Strong winds can lengthen the process and often results in lesser-quality fish that sell at lower prices.

"With the method of iron mesh, our smoked fish is of poor quality, they are sold at 600 to 750 rupees per kilo. This is a very low price. The money we earn is not even enough to send our children to school," said Fransis Devamalar, a fish smoker who heads her family of four children.

The FAO technology was first introduced in Africa, where 12 countries have now adopted it. Women using old methods of fish-smoking over an open fire often suffer eye and skin irritations and respiratory disease from the smoke. However, African women who have adopted the new method have improved their health, significantly increased their income, reduced their costs, cut down on losses, improved the quality and safety of their smoked fish, and were able to improve their family's food security and nutrition.



Ms. Kopalapillai Theivamalar makes her living from fish-smoking in Sri Lanka. Using the traditional fish-smoking method can cause health hazards from smoke inhalation.

> The first thing I noticed was that the new technology helps me save time,"- said Kopalapillai Theivarmallar who makes her living from fish-smoking to provide for her three daughters. "Before, I spent a total of 12 hours on two consecutive days drying and smoking the fish, and I used the old method of iron mesh.

Fig. 8.4: Media report[7] in Sri Lanka (8 December, 2017) on the introduction of the FTT in that country, emphasizing the positive impact of the innovation on the health and livelihoods of processors, without demonizing the traditional smoking ovens. This is a positive example of risk communication on traditional and improved ovens

[7]Story available online at: https://www.pressreader.com/sri-lanka/sunday-times-sri-lanka/20171210/282789241779856 , https://www.dailynews.lk/2017/12/08/business/136699/innovation-fish-smoking-improves-lives

8.9 Dissemination of study findings

The results of this study have been shared via several means including a webinar, training workshops for fish processors, and in policy support documents written with and/or for the UN Food and Agriculture organization (FAO) during the research period. In addition to standard scientific communication (e.g. paper publishing and conference presentations), it was important to bring the findings home to the stakeholders who, in the end, operationalize the results of scientific inquiry. Details are provided below:

- **Webinar in Rome**: On 16th September, 2016, a webinar was organized at the FAO Headquarters in Rome through the organization's Food Safety Technical Network, specifically for sharing the results of the comparative smoking experiments between the FTT and the traditional ovens vis-à-vis PAH contamination levels and consumer exposure. Participants were drawn from several regions of the world with FAO representation. The promoters of this PhD study were also present in Rome for the event. The webinar was chaired by the Head of the Food Safety and Quality Unit of the Agriculture and Consumer Protection Department of the FAO. The presentation was well-received and occasioned additional support from the FAO for an extended coverage of the data collection beyond the Greater Accra Region of Ghana, and for the sensory evaluation of the products.

- **Chemical risk assessment training in Ghana**: From 30th November to 2nd December, 2016, the PAH contamination and consumer exposure data were used to train staff of the Food and Drugs Authority of Ghana (FDA), the Ghana Standards Authority (GSA), and the Food Research Institute of the Council for Scientific and Industrial Research (CSIR-FRI) as a local example of chemical risk assessment.

- **Sustainable small-scall fisheries workshop:** From 20th to 22nd June, 2017, a workshop was organized in Ho in the Volta Region of Ghana for a country-specific validation of the Voluntary Guidelines for Securing Sustainable Small-scale Fisheries in the Context of Food Security and Poverty Eradication (SSF

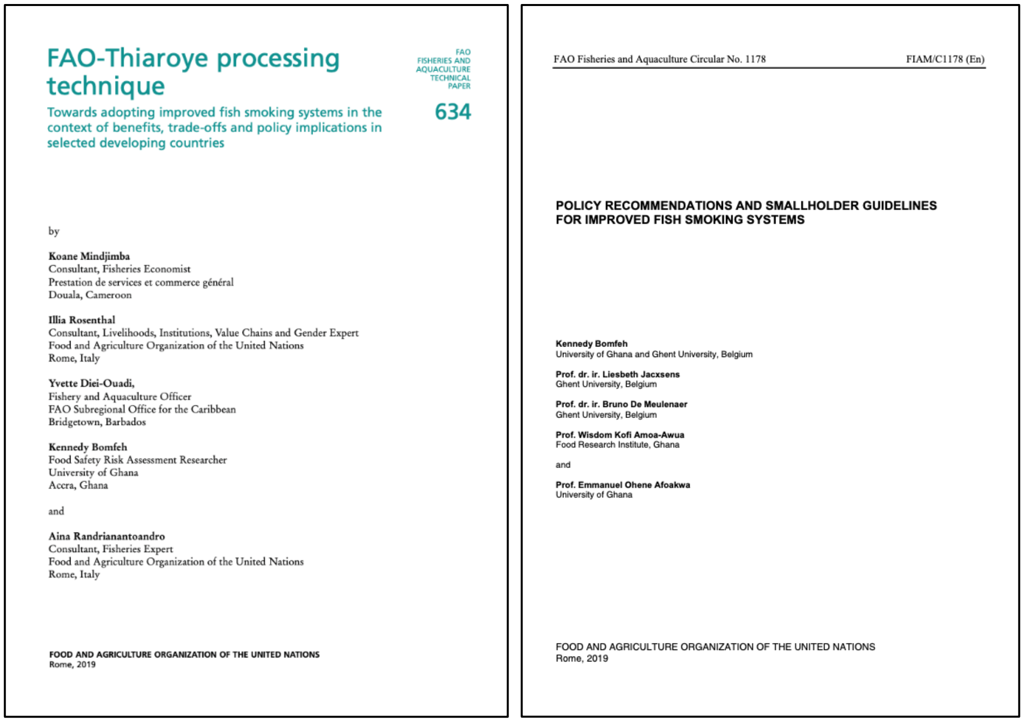
Guidelines). At that meeting, the study findings were shared to provide the participants with smoking-oven related food safety insights. The participants were stakeholders in the fisheries value chain including fisherfolk, fish processors, government agencies, non- and intergovernmental agency representatives and researchers.

- **Results dissemination workshop in Accra, Ghana**: A workshop was organized in Accra, on 8th September, 2017, to share the study findings with stakeholders in the fisheries value chain in Ghana. Participants were drawn from the Food and Drugs Authority of Ghana (FDA), the Ghana Standards Authority (GSA), the Food Research Institute of the Council for Scientific and Industrial Research, Ghana, (CSIR-FRI), The Fisheries Commission of the Ministry of Fisheries and Aquaculture Development, the National Fish Processors and Traders Association of Ghana (NAFPTA), the FAO Regional Office for Africa, researchers and University students, and the general public. The findings were widely circulated on Ghana's media platforms (e.g. in Fig. 8.2). The promoters of this PhD study were also present in Ghana for the programme.

- **Training for an association of women fish processors in Ghana**: From 18th – 19th of February, 2019, a training was organized for women fish processors in Accra on the benefits, use and maintenance of the FTT (Fig. 8.4). Participants experienced and appreciated the benefits of the FTT, especially the reduced occupational exposure to smoke during use, and were given practical exposure to how the components of the FTT reduce PAH contamination in smoked fish.

Fig. 8.4: Group photograph with an association of women fish processors after a training session on the features, use and maintenance of the FTT in Ghana. PhD candidate first from left.

- **Publication of policy support documents**: Two FAO policy support documents were published using (some) results from the study (Fig. 8.5). The wide readership of FAO documents will be significant in ensuring a far-reaching dissemination of the findings, especially in the South.


FAO-Thiaroye processing technique
Towards adopting improved fish smoking systems in the context of benefits, trade-offs and policy implications in selected developing countries

FAO FISHERIES AND AQUACULTURE TECHNICAL PAPER
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Rome, 2019

FAO Fisheries and Aquaculture Circular No. 1178 FIAM/C1178 (En)

POLICY RECOMMENDATIONS AND SMALLHOLDER GUIDELINES FOR IMPROVED FISH SMOKING SYSTEMS

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Rome, 2019

Fig. 8.5: FAO policy support documents written in part (left) and in full (right) based on the findings of this study

- **Expert meeting of the African Network of Fish Technology and Safety (ANFTS)**: In November 2017, the study findings were shared at the ANFTS in Elmina, Ghana. The meetings serve as an important rallying point for research and development regarding food safety, technology and marketing in fisheries in Africa and has a history of over three decades.

- **Regional capacity building for women and youth on the use of the (FTT)** This meeting was held in Elmina, Ghana, from 25^{th} – 27th September, 2019. The workshop was jointly organized by the Products, Trade and Marketing Branch (FIAM) of the Fisheries and Aquaculture Department of FAO Headquarters, the FAO Regional Office for Africa (FAO- RAF) and WorldFish [under The Technology for Africa Agriculture Transformation (TAAT) Program]. It brought together 59 participants from 28 countries from Africa and Asia. The findings of this study were shared, and significant contributions made by the PhD candidate towards revision of the manual for the construction and use of the FTT.

8.10 Conclusions and recommendations for future research

The presented study results suggest that PAHs are the principal hazards in traditionally smoked fish in Ghana, occurring at elevated levels due to the use of the Chorkor smoker and the metal drum oven for fish smoking. The high hazard levels translate into public health concerns of high PAH exposure in smoked fish (due to a combination of high contaminant levels and high smoked fish consumption), requiring a risk management action. The FTT has been demonstrated as a technically viable intervention whose products have acceptable sensory attributes to consumers. However, the oxidative stability of unsaturated fatty acids in FTT products is compromised under traditional storage conditions, and introduction of the oven may require more upfront financial inputs than continued use of the traditional ovens. Therefore, a fine balance needs to be struck between protecting public health and protecting the public purse, as far as the adoption and use of the FTT is concerned.

To reduce the construction cost of the FTT, the removal of the indirect smoke generator from the oven design is recommended, as that appendage does not offer any additional benefit to reducing PAHs when the recommended fuel is used along with the fat collector.

For future research, it is recommended that some fundamental studies be conducted on the physicochemical properties and microbiological quality of hot-smoked fish and how these evolve from the raw fish through processing, storage, sales and at the point of consumption. Such a study should also highlight any differences in the aforementioned characteristics that may be attributable to the oven type used. Other recommended studies are:

- the evaluation of the impact of processing time-temperature regimes on smoked fish quality (safety and sensory acceptability);
- assessment of the deposition of phenolic compounds on fish during smoking and its relationship with the type of processing fuel, oxidative stability of fish lipids, microbial profile and shelf life of the products;

- evaluation of the flavour profile of smoked fish, considering the effects of fish species, product type (smoked-soft or smoked dry), oven type, storage duration, and consumer responses to such profiles; and
- quantitative determination of the occurrence of oxidized fatty acid species in smoked fish as influenced by fish species, smoked product type, oven type, processing temperature and storage.

Finally, improving the presented MCDA based on evidence from stakeholder engagements in the fisheries value chain is recommended to arrive at a compromise decision that adequately and transparently reflects the needs in the Ghanaian context.

APPENDICES

Appendix 1: Benzo(a)anthracene, chrysene and benzo(b)fluoranthene levels in products

Appendix 1A: Benzo(a)anthracene, chrysene and benzo(b)fluoranthene levels (mean±stdev) in *Sphyraena* sp. and *Sardinella* sp. smoked on three different ovens. (Experiment Set I: FTT vs. traditional ovens as systems for fish smoking) (n=5 for each product)

		Concentration of PAH (mean±stdev)		
Product	**Oven**	**Benzo(a)anthracene**	**Chrysene**	**Benzo(b)fluoranthene**
Smoked-soft *Sphyraena* sp. (n=5)	Chorkor	75 ± 2	94 ± 4	51.3 ± 0.9
	Metal drum	51 ± 13	44 ± 11	34 ± 7.
	FTT	1.1 ± 0.3	1.3 ± 0.3	0.6 ± 0.3
Smoked-soft *Sphyraena* sp. (n=5)	Chorkor	105 ± 8	123 ± 12	72 ± 8
	Metal drum	114 ± 1	103 ± 2	62.5 ± 0.6
	FTT	2 ± 1	2 ± 1	1.2 ± 0.7
Smoked-soft *Sardinella* sp. (n=5)	Chorkor	45.2 ± 0.4	67.1 ± 0.6	28.1 ± 0.7
	Metal drum	37 ± 3	48 ± 4	25 ± 1
	FTT	0.6 ± 0.3	0.8 ± 0.3	0.5 ± 0.2
Smoked-dried *Sardinella* sp. (n=5)	Chorkor	115 ± 3	148 ± 2	72 ± 1
	Metal drum	41.4 ± 0.7	52.2 ± 0.6	26.8 ± 0.3
	FTT	0.4 ± 0	0.6 ± 0.1	0.3 ± 0.1
Raw *Sphyraena* sp. (n=5)	-	<0.2	<0.2	<0.2
Raw *Sardinella* sp. (n=5)	-	<0.20	<0.2	<0.2

Fuel: Chorkor smoker and Metal drum - *Pterocarpus erinaceous*; FTT – charcoal for cooking, sugarcane bagasse for flavouring

Appendix 1B: Benzo(a)anthracene, chrysene and benzo(b)fluoranthene levels (mean±stdev) in smoked-dry *Sardinella* sp. smoked on three different ovens. (Experiment Set 2: Effect of fuel type on PAH levels; FTT vs. traditional ovens) (n=5 for each oven)

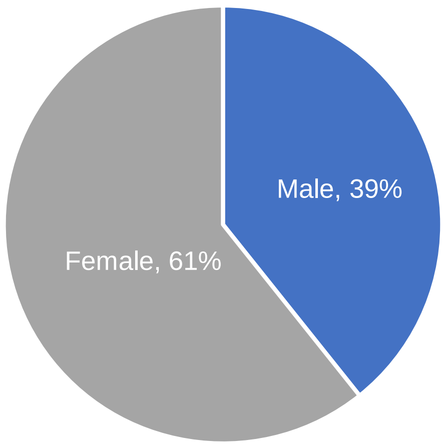
Oven	Fuel	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene
Chorkor smoker	*Pterocarpus erinaceous*	1154 ± 3	147 ± 2	72 ± 1
	Azadirachta indica	48 ± 5	67 ± 5	34 ± 6
	Charcoal for cooking, sugarcane bagasse for flavouring	2.8 ± 0.04	17 ± 1	8.9 ± 0.7
Metal Drum	*Pterocarpus erinaceous*	41.4 ± 0.7	52.2 ± 0.6	26.8 ± 0.3
	Azadirachta indica	48 ± 7	57 ± 10	33 ± 5
FTT	Charcoal+stones for cooking, sugarcane bagasse for flavouring	0.6 ± 0.3	0.7 ± 0.3	0.47 ± 0.17
	Pterocarpus erinaceous	9 ± 2	17 ± 4	9.4 ± 0.6
	Azadirachta indica	6.0 ± 0.5	8 ± 1	7.2 ± 0.4

Appendix 1C: Benzo(a)anthracene, chrysene and benzo(b)fluoranthene levels (mean±stdev) in smoked-dry *Sardinella* sp. smoked on the FTT (Experiment Set 3: Effect of FTT parts on PAH levels) (n=5 for each test condition)

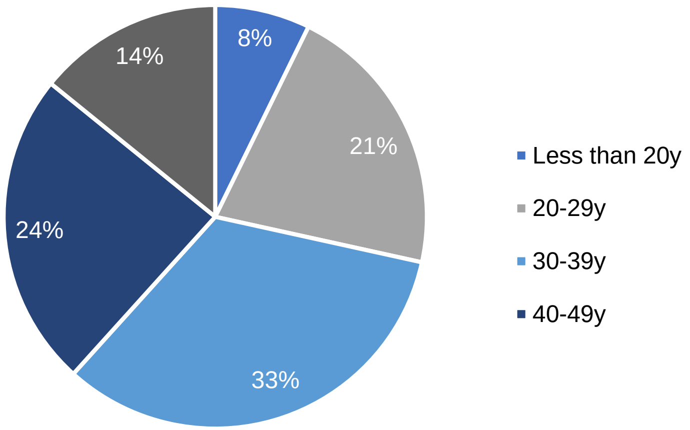
Test		**Condition**	**Benzo(a)anthracene**	**Chrysene**	**Benzo(b)fluoranthene**
Effect of fat collector		Used	1.2 ± 0.1	2.0 ± 0.1	1.1 + 0.0
		Not used	10.0 ± 0.8	17.3 ± 1.0	9.2 ± 0.8
Effect of smoke flavouring material		Sugarcane bagasse	0.4 ± 0.0	0.6 ± 0.1	0.3 ± 0.1
		Coconut husk	0.6 ± 0.5	0.7 ± 0.5	0.5 ± 0.4
		A. indica	2.0 ± 0.7	4.1 ± 0.9	1.5 ± 0.4
Effect of filter	Sugarcane bagasse as smoke material	Filer used	0.4 ± 0.0	0.6 ± 0.1	0.3 ± 0.1
		Filer not used	2.1 ± 0.1	3.5 ± 0.2	1.5 ± 0.1
	A. indica as smoke material	Used	2.0 ± 0.7	4.1 ± 0.9	1.5 ± 0.4
		Not used	3.4 ± 0.2	5.9 ± 0.2	1.9 ± 0.8

Appendix 2: Demographic information of respondents in the smoked-dry *Sardinella* sp. consumption survey

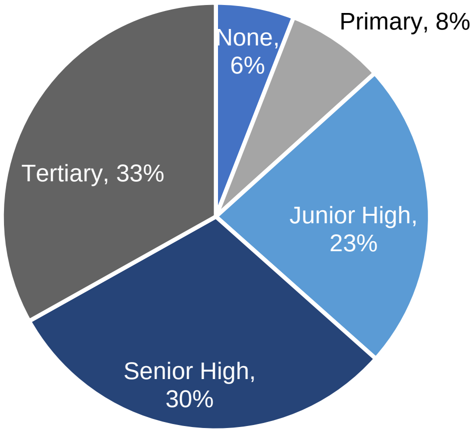
Overall, more females (61%) than males (39%) participated in the survey. The majority were aged between 30-39 years (33%), and had tertiary level education (30%) (Appendix 2a – 2c). Demographic information of respondents in each region is shown in Appendix 2d.



Appendix 2a: Gender of respondents across the survey regions regions



Appendix 2b: Gender of respondents across the survey regions regions

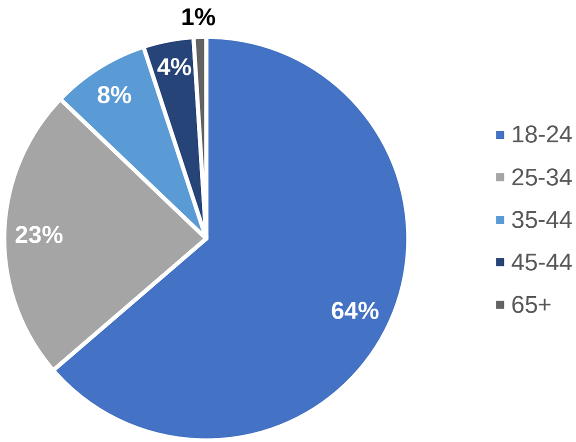


Appendix 2c: Educational level of respondents across regions

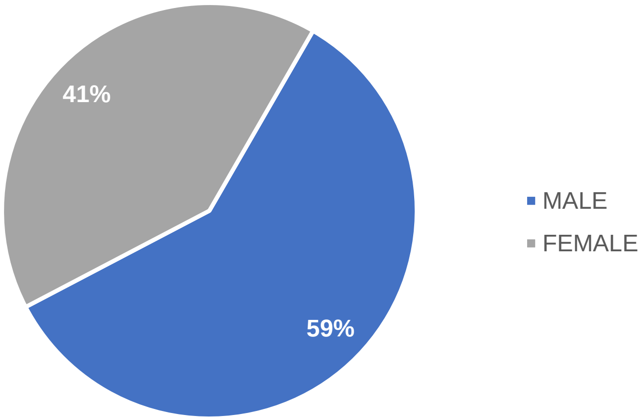
Appendix 2d: Summary of demographic information of respondents in a consumer survey on intakes of smoked-dry *Sardinella* sp. among three regions in Ghana

Demographic		Number of respondents		
		Greater Accra Region (n=212)	Brong Ahafo Region (n=300)	Upper East Region (n=300)
Gender	Male	76	115	128
	Female	136	185	172
Age	Less than 20y	21	21	17
	20-29y	50	42	80
	30-39y	70	85	115
	40-49y	39	94	63
	50+	32	58	25
Level of Education	None	30	1	17
	Primary	16	20	24
	Junior High School	50	57	82
	High School	64	128	54
	Tertiary	52	94	123

Appendix 3: Age and gender distribution of respondents in the consumer acceptance test in Ghana



Appendix 3a: Age distribution of respondents in a consumer acceptance test of smoked mackerel processed on the FTT vs. the Chorkokor smoker in Ghana (n=90)



Appendix 3b: Gender distribution of respondents in a consumer acceptance test of smoked mackerel processed on the FTT vs. the Chorkokor smoker in Ghana (n=90)

Appendix 4: Fatty acid profile of decapsulated fish oil

Fatty acid group		Fatty acid	Krill W0	Krill W3	Krill T	Biover W0	Biover W3	Biover T	Boni W0	Boni W3	Boni T	Vitarmonyl W0	Vitarmonyl W3	Vitarmonyl T	Lucovitaal W0	Lucovitaal W3	Lucovitaal T	Kruidvat W0	Kruidvat W3	Kruidvat T
SFA (g/100g)	C14:0	Myristic acid	6.3	6.2	6.6	nd	nd	nd	0.1	nd	nd	0.4	0.4	0.3	0.1	0.1	0.1	5.0	4.4	4.5
	C16:0	Palmitic acid	15.4	14.6	15.8	0.5	0.4	0.4	0.9	0.8	0.8	1.7	1.7	1.5	2.1	2.1	2.0	12.5	11.2	11.5
	C18:0	Stearic acid	1.3	1.2	1.3	3.3	3.0	3.1	3.7	3.4	3.4	2.5	2.5	2.2	3.7	3.5	3.4	2.9	2.5	2.6
	C20:0	Arachidic acid	0.1	0.9	0.1	0.4	0.8	0.9	0.9	0.8	0.4	0.6	0.6	0.3	0.7	0.8	0.8	0.5	0.6	0.5
	C22:0	Behenic acid	nd	nd	nd	0.3	0.2	0.1	0.3	0.3	0.3	0.4	0.4	0.2	0.3	0.2	0.2	0.2	0.2	0.1
	C24:0	Lignoceric acid	0.1	0.1	0.3	0.1	0.1	0.1	0.1	nd	0.4	0.2	0.1	0.4	0.1	nd	0.2	nd	nd	0.2
	Total		**23.2**	**23.0**	**24.1**	**4.6**	**4.5**	**4.5**	**5.8**	**5.4**	**5.3**	**5.9**	**5.7**	**5.0**	**7.0**	**6.7**	**6.6**	**21.2**	**18.9**	**19.4**
MUFA(g/100g)	C14:1	Myristoleic acid	0.1	0.1	0.1	nd	nd	nd	nd	nd	nd	0.04	0.03	nd	nd	nd	nd	0.4	0.1	0.1
	C16:1	Palmitoleic acid	6.1	6.3	6.7	0.4	0.3	0.3	0.5	0.4	0.4	1.1	1.0	0.9	0.6	0.6	0.6	6.2	5.7	5.6
	C18:1c9	Oleic acid	7.3	7.2	7.4	6.7	6.2	6.3	8.0	7.2	6.5	7.5	7.1	5.5	8.5	7.7	6.7	20.3	20.3	19.6
	C18:1c11	*cis*-Vaccenic acid	4.2	4.1	4.6	2.8	2.5	2.6	2.4	2.3	2.6	1.8	2.1	2.4	2.2	2.5	2.6	2.5	0.1	0.2
	C20:1	Gondoic acid	0.7	0.6	0.7	1.7	1.5	1.7	2.1	1.9	1.7	1.7	1.9	1.7	1.5	1.8	1.6	1.8	1.3	1.8
	C22:1	Erucic acid	0.6	0.3	0.7	1.6	1.4	1.5	0.2	1.3	1.5	0.7	0.9	2.2	0.6	0.6	0.5	1.4	1.1	1.1
	C24:1	Nervonic acid	nd	nd	nd	0.3	nd	0.2	0.3	nd	0.2	0.6	0.2	0.2	0.4	/	0.2	0.3	/	0.2
	Total		**18.9**	**18.5**	**20.2**	**13.5**	**11.9**	**12.6**	**13.5**	**13.2**	**12.9**	**13.5**	**13.3**	**12.8**	**13.7**	**13.2**	**12.2**	**33.0**	**28.7**	**28.6**
PUFA (g/100g)	C18:2 ω6	Linoleic acid	0.8	0.7	0.8	1.4	1.3	1.2	1.2	1.1	0.9	1.3	1.3	1.0	12.7	12.1	9.6	5.8	5.1	4.7
	C18:3 ω3	Linolenic acid	0.5	0.5	0.5	0.9	0.9	0.8	0.8	0.8	0.7	0.7	0.7	0.5	0.7	0.7	0.6	2.3	2.2	1.5
	C20:2 ω6	Eicosadienoic acid	1.9	1.9	1.5	2.1	1.9	1.6	2.1	1.9	1.1	1.5	1.5	0.7	1.8	1.7	1.0	2.3	2.0	1.2
	C20:3 ω6	Dihomogammalinolenic acid	nd	nd	nd	0.1	0.2	0.1	0.2	0.2	nd	0.2	0.2	nd	0.2	0.2	0.1	0.2	0.2	0.2
	C20:4	Arachidonic acid	0.2	0.2	0.2	2.1	1.9	1.5	1.5	1.4	0.8	1.9	1.9	0.8	1.6	1.5	0.7	0.7	0.6	0.4
	C20:5 ω3	Eicosapentaenoic acid	15.7	15.6	11.9	30.1	26.9	20.4	28.2	25.6	10.5	26.1	25.4	0.0	23.7	22.3	9.0	10.9	9.6	4.1
	C22:6 ω3	Docosahexaenoic acid	5.7	5.6	3.1	19.7	18.0	12.7	20.4	19.0	4.7	17.0	16.5	3.3	15.4	14.4	3.8	8.7	7.7	2.2
	Total		**24.8**	**24.4**	**18.0**	**56.6**	**51.0**	**38.3**	**54.4**	**50.2**	**18.7**	**48.7**	**47.6**	**6.4**	**56.1**	**52.9**	**24.8**	**30.9**	**27.3**	**14.4**
	Unidentified fatty acids (g/100g		**6.2**	**3.9**	**5.6**	**6.9**	**4.5**	**4.2**	**6.5**	**4.5**	**5.4**	**7.8**	**6.7**	**4.3**	**5.5**	**6.2**	**4.5**	**7.7**	**7.0**	**5.4**
Total fatty acids (g/100g of capsule oil)			**74.2**	**70.9**	**68.6**	**86.6**	**77.5**	**63.9**	**86**	**78.3**	**44.4**	**82.3**	**79.5**	**38.7**	**86.8**	**82**	**49.8**	**95.4**	**84.2**	**68.6**

nd = not detected
Values are single measurements (no replicates)
W0 = Week 0 storage; W3 = Week three storage; T = Thermoxidized

Appendix 5: Two-way ANOVA summary tables

Appendix 5A: Comparison of moisture contents of smoked-dry Sardinella *sp. produced on three different ovens and stored for up to 6 months*

Table 5A (1): Testing impact of oven type on the moisture contents of smoked-dry Sardinella sp. stored for the same duration

Storage Month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	4.796*	,271	,000	4,246	5,346
		Metal drum oven	3.400*	,271	,000	2,850	3,950
	Chorkor smoker	FTT	-4.796*	,271	,000	-5,346	-4,246
		Metal drum oven	-1.396*	,271	,000	-1,946	-,846
	Metal drum oven	FTT	-3.400*	,271	,000	-3,950	-2,850
		Chorkor smoker	1.396*	,271	,000	,846	1,946
Month 3	FTT	Chorkor smoker	,216	,271	,431	-,334	,766
		Metal drum oven	,106	,271	,698	-,444	,656
	Chorkor smoker	FTT	-,216	,271	,431	-,766	,334
		Metal drum oven	-,110	,271	,687	-,660	,440
	Metal drum oven	FTT	-,106	,271	,698	-,656	,444
		Chorkor smoker	,110	,271	,687	-,440	,660
Month 6	FTT	Chorkor smoker	.780*	,271	,007	,230	1,330
		Metal drum oven	.778*	,271	,007	,228	1,328
	Chorkor smoker	FTT	-.780*	,271	,007	-1,330	-,230
		Metal drum oven	-,002	,271	,994	-,552	,548
	Metal drum oven	FTT	-.778*	,271	,007	-1,328	-,228
		Chorkor smoker	,002	,271	,994	-,548	,552

*. The mean difference is significant at the .05 level.

Table 5A (2) : Testing impact of storage month on the moisture contents of smoked-dry Sardineall sp. from the same oven

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
FTT	Month 0	Month 3	4.500*	,271	,000	3,950	5,050
		Month 6	3.096*	,271	,000	2,546	3,646
	Month 3	Month 0	-4.500*	,271	,000	-5,050	-3,950
		Month 6	-1.404*	,271	,000	-1,954	-,854
	Month 6	Month 0	-3.096*	,271	,000	-3,646	-2,546
		Month 3	1.404*	,271	,000	,854	1,954
Chorkor smoker	Month 0	Month 3	-,080	,271	,770	-,630	,470
		Month 6	-.920*	,271	,002	-1,470	-,370
	Month 3	Month 0	,080	,271	,770	-,470	,630
		Month 6	-.840*	,271	,004	-1,390	-,290
	Month 6	Month 0	.920*	,271	,002	,370	1,470
		Month 3	.840*	,271	,004	,290	1,390
Metal drum oven	Month 0	Month 3	1.206*	,271	,000	,656	1,756
		Month 6	,474	,271	,089	-,076	1,024
	Month 3	Month 0	-1.206*	,271	,000	-1,756	-,656
		Month 6	-.732*	,271	,010	-1,282	-,182
	Month 6	Month 0	-,474	,271	,089	-1,024	,076
		Month 3	.732*	,271	,010	,182	1,282

*. The mean difference is significant at the .05 level.

Appendix 5B: Comparison of fat contents of smoked-dry Sardinella *sp. produced on three different ovens and stored for up to 6 months. Fat contents compared on g/100g dry matter of smoked fish.*

Table 5B (1) : Testing impact of oven type on the fat contents of smoked-dry *Sardinella* sp. stored for the same duration (smoked fish dry matter basis)

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Month 0	FTT	Chorkor	-,664	,690	,342	-2,064	,736
		Metal drum	-6.262*	,690	,000	-7,662	-4,862
	Chorkor	FTT	,664	,690	,342	-,736	2,064
		Metal drum	-5.598*	,690	,000	-6,998	-4,198
	Metal drum	FTT	6.262*	,690	,000	4,862	7,662
		Chorkor	5.598*	,690	,000	4,198	6,998
Month 3	FTT	Chorkor	-,820	,690	,243	-2,220	,580
		Metal drum	-4.100*	,690	,000	-5,500	-2,700
	Chorkor	FTT	,820	,690	,243	-,580	2,220
		Metal drum	-3.280*	,690	,000	-4,680	-1,880
	Metal drum	FTT	4.100*	,690	,000	2,700	5,500
		Chorkor	3.280*	,690	,000	1,880	4,680
Month 6	FTT	Chorkor	-1.580*	,690	,028	-2,980	-,180
		Metal drum	-3.160*	,690	,000	-4,560	-1,760
	Chorkor	FTT	1.580*	,690	,028	,180	2,980
		Metal drum	-1.580*	,690	,028	-2,980	-,180
	Metal drum	FTT	3.160*	,690	,000	1,760	4,560
		Chorkor	1.580*	,690	,028	,180	2,980

*. The mean difference is significant at the .05 level.

Table 5B (2) : Testing impact of storage time on the fat contents of smoked-dry *Sardinella* sp. produced on the same oven (smoked fish dry matter basis)

Oven	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
FTT	Month 0	Month 3	1,362	,690	,056	-,038	2,762
		Month 6	1,042	,690	,140	-,358	2,442
	Month 3	Month 0	-1,362	,690	,056	-2,762	,038
		Month 6	-,320	,690	,646	-1,720	1,080
	Month 6	Month 0	-1,042	,690	,140	-2,442	,358
		Month 3	,320	,690	,646	-1,080	1,720
Chorkor	Month 0	Month 3	1,206	,690	,089	-,194	2,606
		Month 6	,126	,690	,856	-1,274	1,526
	Month 3	Month 0	-1,206	,690	,089	-2,606	,194
		Month 6	-1,080	,690	,126	-2,480	,320
	Month 6	Month 0	-,126	,690	,856	-1,526	1,274
		Month 3	1,080	,690	,126	-,320	2,480
Metal drum	Month 0	Month 3	3.524*	,690	,000	2,124	4,924
		Month 6	4.144*	,690	,000	2,744	5,544
	Month 3	Month 0	-3.524*	,690	,000	-4,924	-2,124
		Month 6	,620	,690	,375	-,780	2,020
	Month 6	Month 0	-4.144*	,690	,000	-5,544	-2,744
		Month 3	-,620	,690	,375	-2,020	,780

*. The mean difference is significant at the .05 level.

Appendix 5C: Comparison of saturated fatty acid content (SFA) of smoked-dry Sardinella *sp. produced on three different ovens and stored for up to 6 months. SFA compared on g/100g of extracted fat*

Table 5C (1): Testing impact of storage time on the SFA contents of smoked-dry *Sardinella* sp. produced on the same oven (extracted fat basis)

Oven	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
FTT	Month 0	Month 3	1,308	,846	,132	-,414	3,029
		Month 6	1,610	,846	,066	-,112	3,331
	Month 3	Month 0	-1,308	,846	,132	-3,029	,414
		Month 6	,302	,798	,708	-1,321	1,925
	Month 6	Month 0	-1,610	,846	,066	-3,331	,112
		Month 3	-,302	,798	,708	-1,925	1,321
Chorkor smoker	Month 0	Month 3	-,614	,798	,447	-2,237	1,009
		Month 6	-1,245	,846	,151	-2,966	,477
	Month 3	Month 0	,614	,798	,447	-1,009	2,237
		Month 6	-,631	,846	,462	-2,352	1,091
	Month 6	Month 0	1,245	,846	,151	-,477	2,966
		Month 3	,631	,846	,462	-1,091	2,352
Metal Drum	Month 0	Month 3	-1.808*	,798	,030	-3,431	-,185
		Month 6	-4.347*	,846	,000	-6,069	-2,625
	Month 3	Month 0	1.808*	,798	,030	,185	3,431
		Month 6	-2.539*	,846	,005	-4,261	-,817
	Month 6	Month 0	4.347*	,846	,000	2,625	6,069
		Month 3	2.539*	,846	,005	,817	4,261

*. The mean difference is significant at the .05 level.

Table 5C(2) : Testing impact of oven type on the SFA contents of smoked-dry *Sardinella* sp. stored for the same duration (extracted fat basis)

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	,380	,846	,657	-1,342	2,101
		Metal Drum	-1,311	,846	,131	-3,032	,411
	Chorkor smoker	FTT	-,380	,846	,657	-2,101	1,342
		Metal Drum	-1.690*	,798	,042	-3,313	-,067
	Metal Drum	FTT	1,311	,846	,131	-,411	3,032
		Chorkor smoker	1.690*	,798	,042	,067	3,313
Month 3	FTT	Chorkor smoker	-1,542	,798	,062	-3,165	,081
		Metal Drum	-4.426*	,798	,000	-6,049	-2,803
	Chorkor smoker	FTT	1,542	,798	,062	-,081	3,165
		Metal Drum	-2.884*	,798	,001	-4,507	-1,261
	Metal Drum	FTT	4.426*	,798	,000	2,803	6,049
		Chorkor smoker	2.884*	,798	,001	1,261	4,507
Month 6	FTT	Chorkor smoker	-2.475*	,846	,006	-4,196	-,753
		Metal Drum	-7.267*	,846	,000	-8,989	-5,545
	Chorkor smoker	FTT	2.475*	,846	,006	,753	4,196
		Metal Drum	-4.792*	,892	,000	-6,608	-2,977
	Metal Drum	FTT	7.267*	,846	,000	5,545	8,989
		Chorkor smoker	4.792*	,892	,000	2,977	6,608

*. The mean difference is significant at the .05 level.

Appendix 5D: Comparison of the saturated fatty acid content (SFA) of smoked-dry Sardinella *sp. produced on three different ovens and stored for up to 6 months. SFA compared on g/100g of dry matter of smoked fish.*

Table 5D (1) : Testing impact of oven type on the SFA contents of smoked-dry *Sardinella* sp. stored for the same duration (smoked fish dry matter basis)

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	-.435*	,165	,013	-,772	-,098
		Metal Drum	-2.034*	,157	,000	-2,353	-1,715
	Chorkor smoker	FTT	.435*	,165	,013	,098	,772
		Metal Drum	-1.599*	,157	,000	-1,918	-1,280
	Metal Drum	FTT	2.034*	,157	,000	1,715	2,353
		Chorkor smoker	1.599*	,157	,000	1,280	1,918
Month 3	FTT	Chorkor smoker	-.316*	,148	,040	-,617	-,015
		Metal Drum	-1.574*	,148	,000	-1,875	-1,273
	Chorkor smoker	FTT	.316*	,148	,040	,015	,617
		Metal Drum	-1.258*	,148	,000	-1,559	-,957
	Metal Drum	FTT	1.574*	,148	,000	1,273	1,875
		Chorkor smoker	1.258*	,148	,000	,957	1,559
Month 6	FTT	Chorkor smoker	-,255	,165	,132	-,592	,082
		Metal Drum	-1.465*	,165	,000	-1,802	-1,128
	Chorkor smoker	FTT	,255	,165	,132	-,082	,592
		Metal Drum	-1.210*	,165	,000	-1,547	-,873
	Metal Drum	FTT	1.465*	,165	,000	1,128	1,802
		Chorkor smoker	1.210*	,165	,000	,873	1,547

*. The mean difference is significant at the .05 level.

Table 5D (2) : Testing impact of storage time on the SFA contents of smoked-dry *Sardinella* sp. produced on the same oven (smoked fish dry matter basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
FTT	Month 0	Month 3	.366*	,157	,026	,047	,685
		Month 6	,215	,165	,202	-,122	,552
	Month 3	Month 0	-.366*	,157	,026	-,685	-,047
		Month 6	-,151	,157	,342	-,470	,168
	Month 6	Month 0	-,215	,165	,202	-,552	,122
		Month 3	,151	,157	,342	-,168	,470
Chorkor smoker	Month 0	Month 3	.485*	,157	,004	,166	,804
		Month 6	.395*	,165	,023	,058	,732
	Month 3	Month 0	-.485*	,157	,004	-,804	-,166
		Month 6	-,090	,157	,570	-,409	,229
	Month 6	Month 0	-.395*	,165	,023	-,732	-,058
		Month 3	,090	,157	,570	-,229	,409
Metal Drum	Month 0	Month 3	.826*	,148	,000	,525	1,127
		Month 6	.784*	,157	,000	,465	1,103
	Month 3	Month 0	-.826*	,148	,000	-1,127	-,525
		Month 6	-,042	,157	,790	-,361	,277
	Month 6	Month 0	-.784*	,157	,000	-1,103	-,465
		Month 3	,042	,157	,790	-,277	,361

*. The mean difference is significant at the .05 level.

Appendix 5E: Comparison of the monounsaturated fatty acid (MUFA) content of smoked-dry Sardinella *sp. produced on three different ovens and stored for up to 6 months. MUFA compared on g/100g of extracted fat of smoked fish*

Table 5E(1) : Testing impact of storage time on the MUFA contents of smoked-dry *Sardinella* sp. produced on the same oven (extracted fat basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
FTT	Month 0	Month 3	,890	,477	,071	-,081	1,860
		Month 6	,640	,477	,189	-,331	1,610
	Month 3	Month 0	-,890	,477	,071	-1,860	,081
		Month 6	-,250	,450	,582	-1,165	,665
	Month 6	Month 0	-,640	,477	,189	-1,610	,331
		Month 3	,250	,450	,582	-,665	1,165
Chorkor smoker	Month 0	Month 3	-,072	,450	,874	-,987	,843
		Month 6	-,812	,477	,098	-1,782	,158
	Month 3	Month 0	,072	,450	,874	-,843	,987
		Month 6	-,740	,477	,130	-1,710	,230
	Month 6	Month 0	,812	,477	,098	-,158	1,782
		Month 3	,740	,477	,130	-,230	1,710
Metal Drum	Month 0	Month 3	,354	,450	,437	-,561	1,269
		Month 6	-,773	,477	,115	-1,743	,198
	Month 3	Month 0	-,354	,450	,437	-1,269	,561
		Month 6	-1.127*	,477	,024	-2,097	-,156
	Month 6	Month 0	,773	,477	,115	-,198	1,743
		Month 3	1.127*	,477	,024	,156	2,097

*. The mean difference is significant at the .05 level.

Table 5E(2) : Testing impact of oven type on the MUFA contents of smoked-dry *Sardinella* sp. stored for the same duration (extracted fat basis)

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	-,420	,477	,384	-1,391	,550
		Metal Drum	-3.583*	,477	,000	-4,553	-2,612
	Chorkor smoker	FTT	,420	,477	,384	-,550	1,391
		Metal Drum	-3.162*	,450	,000	-4,077	-2,247
	Metal Drum	FTT	3.583*	,477	,000	2,612	4,553
		Chorkor smoker	3.162*	,450	,000	2,247	4,077
Month 3	FTT	Chorkor smoker	-1.382*	,450	,004	-2,297	-,467
		Metal Drum	-4.118*	,450	,000	-5,033	-3,203
	Chorkor smoker	FTT	1.382*	,450	,004	,467	2,297
		Metal Drum	-2.736*	,450	,000	-3,651	-1,821
	Metal Drum	FTT	4.118*	,450	,000	3,203	5,033
		Chorkor smoker	2.736*	,450	,000	1,821	3,651
Month 6	FTT	Chorkor smoker	-1.872*	,477	,000	-2,842	-,902
		Metal Drum	-4.995*	,477	,000	-5,965	-4,024
	Chorkor smoker	FTT	1.872*	,477	,000	,902	2,842
		Metal Drum	-3.123*	,503	,000	-4,145	-2,100
	Metal Drum	FTT	4.995*	,477	,000	4,024	5,965
		Chorkor smoker	3.123*	,503	,000	2,100	4,145

*. The mean difference is significant at the .05 level.

Appendix 5F: Comparison of monounsaturated fatty acid (MUFA) content of smoked-dry Sardinella sp. produced on three different ovens and stored for up to 6 months. MUFA compared on g/100g of dry matter of smoked fish

Table 5F(1): Testing storage time on the MUFA contents of smoked-dry *Sardinella* sp. produced on the same oven (smoked fish dry matter basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
FTT	Month 0	Month 3	,170	,087	,060	-,008	,347
		Month 6	,078	,092	,404	-,109	,264
	Month 3	Month 0	-,170	,087	,060	-,347	,008
		Month 6	-,092	,087	,297	-,269	,085
	Month 6	Month 0	-,078	,092	,404	-,264	,109
		Month 3	,092	,087	,297	-,085	,269
Chorkor smoker	Month 0	Month 3	.216*	,087	,018	,039	,393
		Month 6	,150	,092	,111	-,037	,337
	Month 3	Month 0	-.216*	,087	,018	-,393	-,039
		Month 6	-,066	,087	,453	-,243	,111
	Month 6	Month 0	-,150	,092	,111	-,337	,037
		Month 3	,066	,087	,453	-,111	,243
Metal Drum	Month 0	Month 3	.566*	,082	,000	,399	,733
		Month 6	.546*	,087	,000	,369	,723
	Month 3	Month 0	-.566*	,082	,000	-,733	-,399
		Month 6	-,020	,087	,819	-,197	,157
	Month 6	Month 0	-.546*	,087	,000	-,723	-,369
		Month 3	,020	,087	,819	-,157	,197

*. The mean difference is significant at the .05 level.

Table 5F(2) : Testing impact of oven type on the MUFA contents of smoked-dry *Sardinella* sp. stored for the same duration (smoked fish dry matter basis)

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	-.233*	,092	,016	-,419	-,046
		Metal Drum	-1.259*	,087	,000	-1,436	-1,081
	Chorkor smoker	FTT	.233*	,092	,016	,046	,419
		Metal Drum	-1.026*	,087	,000	-1,203	-,849
	Metal Drum	FTT	1.259*	,087	,000	1,081	1,436
		Chorkor smoker	1.026*	,087	,000	,849	1,203
Month 3	FTT	Chorkor smoker	-.186*	,082	,030	-,353	-,019
		Metal Drum	-.862*	,082	,000	-1,029	-,695
	Chorkor smoker	FTT	.186*	,082	,030	,019	,353
		Metal Drum	-.676*	,082	,000	-,843	-,509
	Metal Drum	FTT	.862*	,082	,000	,695	1,029
		Chorkor smoker	.676*	,082	,000	,509	,843
Month 6	FTT	Chorkor smoker	-,160	,092	,090	-,347	,027
		Metal Drum	-.790*	,092	,000	-,977	-,603
	Chorkor smoker	FTT	,160	,092	,090	-,027	,347
		Metal Drum	-.630*	,092	,000	-,817	-,443
	Metal Drum	FTT	.790*	,092	,000	,603	,977
		Chorkor smoker	.630*	,092	,000	,443	,817

*. The mean difference is significant at the .05 level.

Appendix 5G: Comparison of polyunsaturated fatty acid (PUFA) content of smoked-dry Sardinella sp. produced on three different ovens and stored for up to 6 months. PUFA compared on g/100g of extracted fat of smoked fish

Table 5G(1) : Testing the impact of storage time onteh PUFA content of smoked-dry Sardinella sp. produced on the same oven (extracted fat basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
FTT	Month 0	Month 3	2.124*	,795	,012	,507	3,741
		Month 6	4.870*	,795	0,00000067	3,253	6,487
	Month 3	Month 0	-2.124*	,795	,012	-3,741	-,507
		Month 6	2.746*	,750	,001	1,221	4,271
	Month 6	Month 0	-4.870*	,795	,000	-6,487	-3,253
		Month 3	-2.746*	,750	,001	-4,271	-1,221
Chorkor smoker	Month 0	Month 3	1,286	,750	,096	-,239	2,811
		Month 6	1,017	,795	,210	-,600	2,634
	Month 3	Month 0	-1,286	,750	,096	-2,811	,239
		Month 6	-,269	,795	,737	-1,886	1,348
	Month 6	Month 0	-1,017	,795	,210	-2,634	,600
		Month 3	,269	,795	,737	-1,348	1,886
Metal Drum	Month 0	Month 3	-2.230*	,750	,005	-3,755	-,705
		Month 6	-3.653*	,795	,000	-5,270	-2,035
	Month 3	Month 0	2.230*	,750	,005	,705	3,755
		Month 6	-1,423	,795	,083	-3,040	,195
	Month 6	Month 0	3.653*	,795	,000	2,035	5,270
		Month 3	1,423	,795	,083	-,195	3,040

*. The mean difference is significant at the .05 level.

Table 5G(2) : Testing the impact of storage time on teh PUFA content of smoked-dry Sardineall sp. produced on the same oven (extracted fat basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
FTT	Month 0	Month 3	2.124*	,795	,012	,507	3,741
		Month 6	4.870*	,795	0,00000067	3,253	6,487
	Month 3	Month 0	-2.124*	,795	,012	-3,741	-,507
		Month 6	2.746*	,750	,001	1,221	4,271
	Month 6	Month 0	-4.870*	,795	,000	-6,487	-3,253
		Month 3	-2.746*	,750	,001	-4,271	-1,221
Chorkor smoker	Month 0	Month 3	1,286	,750	,096	-,239	2,811
		Month 6	1,017	,795	,210	-,600	2,634
	Month 3	Month 0	-1,286	,750	,096	-2,811	,239
		Month 6	-,269	,795	,737	-1,886	1,348
	Month 6	Month 0	-1,017	,795	,210	-2,634	,600
		Month 3	,269	,795	,737	-1,348	1,886
Metal Drum	Month 0	Month 3	-2.230*	,750	,005	-3,755	-,705
		Month 6	-3.653*	,795	,000	-5,270	-2,035
	Month 3	Month 0	2.230*	,750	,005	,705	3,755
		Month 6	-1,423	,795	,083	-3,040	,195
	Month 6	Month 0	3.653*	,795	,000	2,035	5,270
		Month 3	1,423	,795	,083	-,195	3,040

*. The mean difference is significant at the .05 level.

Appendix 5H: Comparison of polyunsaturated fatty acid (PUFA) content of smoked-dry Sardinella sp. produced on three different ovens and stored for up to 6 months. PUFA compared on g/100g of dry matter of smoked fish

Table 5H(1) : Testing the impact of storage time on the PUFA content of smoked-dry Sardinella sp. produced on the same oven (smoked fish dry matter basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
FTT	Month 0	Month 3	.328*	,120	,010	,083	,572
		Month 6	.408*	,126	,003	,150	,665
	Month 3	Month 0	-.328*	,120	,010	-,572	-,083
		Month 6	,080	,120	,509	-,164	,324
	Month 6	Month 0	-.408*	,126	,003	-,665	-,150
		Month 3	-,080	,120	,509	-,324	,164
Chorkor smoker	Month 0	Month 3	.452*	,120	,001	,207	,696
		Month 6	.413*	,126	,003	,155	,670
	Month 3	Month 0	-.452*	,120	,001	-,696	-,207
		Month 6	-,039	,120	,747	-,283	,205
	Month 6	Month 0	-.413*	,126	,003	-,670	-,155
		Month 3	,039	,120	,747	-,205	,283
Metal Drum	Month 0	Month 3	.376*	,113	,002	,146	,606
		Month 6	.367*	,120	,004	,123	,611
	Month 3	Month 0	-.376*	,113	,002	-,606	-,146
		Month 6	-,009	,120	,941	-,253	,235
	Month 6	Month 0	-.367*	,120	,004	-,611	-,123
		Month 3	,009	,120	,941	-,235	,253

*. The mean difference is significant at the .05 level.

Table 5H(2) : Testing the impact of oven type on the PUFA content of smoked-dry Sardinella sp. stored for the same duration (smoked fish dry matter content basis)

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	-.320*	,126	,016	-,577	-,063
		Metal Drum	-1.035*	,120	,000	-1,279	-,790
	Chorkor smoker	FTT	.320*	,126	,016	,063	,577
		Metal Drum	-.715*	,120	,000	-,959	-,470
	Metal Drum	FTT	1.035*	,120	,000	,790	1,279
		Chorkor smoker	.715*	,120	,000	,470	,959
Month 3	FTT	Chorkor smoker	-,196	,113	,092	-,426	,034
		Metal Drum	-.986*	,113	,000	-1,216	-,756
	Chorkor smoker	FTT	,196	,113	,092	-,034	,426
		Metal Drum	-.790*	,113	,000	-1,020	-,560
	Metal Drum	FTT	.986*	,113	,000	,756	1,216
		Chorkor smoker	.790*	,113	,000	,560	1,020
Month 6	FTT	Chorkor smoker	-.315*	,126	,018	-,572	-,058
		Metal Drum	-1.075*	,126	,000	-1,332	-,818
	Chorkor smoker	FTT	.315*	,126	,018	,058	,572
		Metal Drum	-.760*	,126	,000	-1,017	-,503
	Metal Drum	FTT	1.075*	,126	,000	,818	1,332
		Chorkor smoker	.760*	,126	,000	,503	1,017

*. The mean difference is significant at the .05 level.

Appendix 5I: Comparison of total fatty acid (TFA) content of smoked-dry Sardinella sp. produced on three different ovens and stored for up to 6 months. TFA compared on g/100g of extracted fat of smoked fish

Table 5I(1) : Testing the impact of storage time on the TFA conntent of smoked-dry Sardinella sp. produced on the same oven (extract fat basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
FTT	Month 0	Month 3	5.414*	2,117	,015	1,107	9,721
		Month 6	8.282*	2,117	,000	3,975	12,589
	Month 3	Month 0	-5.414*	2,117	,015	-9,721	-1,107
		Month 6	2,868	1,996	,160	-1,193	6,929
	Month 6	Month 0	-8.282*	2,117	,000	-12,589	-3,975
		Month 3	-2,868	1,996	,160	-6,929	1,193
Chorkor smoker	Month 0	Month 3	1,136	1,996	,573	-2,925	5,197
		Month 6	-,448	2,117	,834	-4,754	3,859
	Month 3	Month 0	-1,136	1,996	,573	-5,197	2,925
		Month 6	-1,584	2,117	,460	-5,890	2,723
	Month 6	Month 0	,448	2,117	,834	-3,859	4,754
		Month 3	1,584	2,117	,460	-2,723	5,890
Metal Drum	Month 0	Month 3	-3,754	1,996	,069	-7,815	,307
		Month 6	-9.152*	2,117	,000	-13,459	-4,846
	Month 3	Month 0	3,754	1,996	,069	-,307	7,815
		Month 6	-5.398*	2,117	,016	-9,705	-1,092
	Month 6	Month 0	9.152*	2,117	,000	4,846	13,459
		Month 3	5.398*	2,117	,016	1,092	9,705

*. The mean difference is significant at the .05 level.

Table 5I(2) : Testing the impact of oven type on the TFA content of smoked-dry Sardinella sp. stored for the same duration (extracted fat basis)

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	,550	2,117	,797	-3,757	4,857
		Metal Drum	-2,870	2,117	,184	-7,177	1,437
	Chorkor smoker	FTT	-,550	2,117	,797	-4,857	3,757
		Metal Drum	-3,420	1,996	,096	-7,481	,641
	Metal Drum	FTT	2,870	2,117	,184	-1,437	7,177
		Chorkor smoker	3,420	1,996	,096	-,641	7,481
Month 3	FTT	Chorkor smoker	-3,728	1,996	,071	-7,789	,333
		Metal Drum	-12.038*	1,996	,000	-16,099	-7,977
	Chorkor smoker	FTT	3,728	1,996	,071	-,333	7,789
		Metal Drum	-8.310*	1,996	,000	-12,371	-4,249
	Metal Drum	FTT	12.038*	1,996	,000	7,977	16,099
		Chorkor smoker	8.310*	1,996	,000	4,249	12,371
Month 6	FTT	Chorkor smoker	-8.180*	2,117	,000	-12,486	-3,873
		Metal Drum	-20.304*	2,117	,000	-24,611	-15,998
	Chorkor smoker	FTT	8.180*	2,117	,000	3,873	12,486
		Metal Drum	-12.125*	2,231	,000	-16,665	-7,585
	Metal Drum	FTT	20.304*	2,117	,000	15,998	24,611
		Chorkor smoker	12.125*	2,231	,000	7,585	16,665

*. The mean difference is significant at the .05 level.

Appendix 5J: Comparison of total fatty acid (TFA) content of smoked-dry Sardinella sp. produced on three different ovens and stored for up to 6 months. TFA compared on g/100g of dry matter of smoked fish

Table 5J(1) : Testing the impact of storage time on the TFA content of smoked-dry Sardinella sp. produced on the same oven (smoked fish dry matter content basis)

Ovens	Storage month		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
FTT	Month 0	Month 3	.989*	,384	,015	,205	1,773
		Month 6	,778	,405	,064	-,049	1,604
	Month 3	Month 0	-.989*	,384	,015	-1,773	-,205
		Month 6	-,211	,384	,586	-,996	,573
	Month 6	Month 0	-,778	,405	,064	-1,604	,049
		Month 3	,211	,384	,586	-,573	,996
Chorkor smoker	Month 0	Month 3	1.262*	,384	,003	,477	2,046
		Month 6	1.095*	,405	,011	,269	1,921
	Month 3	Month 0	-1.262*	,384	,003	-2,046	-,477
		Month 6	-,166	,384	,668	-,951	,618
	Month 6	Month 0	-1.095*	,405	,011	-1,921	-,269
		Month 3	,166	,384	,668	-,618	,951
Metal Drum	Month 0	Month 3	1.936*	,362	,000	1,197	2,675
		Month 6	1.873*	,384	,000	1,089	2,657
	Month 3	Month 0	-1.936*	,362	,000	-2,675	-1,197
		Month 6	-,063	,384	,871	-,847	,721
	Month 6	Month 0	-1.873*	,384	,000	-2,657	-1,089
		Month 3	,063	,384	,871	-,721	,847

*. The mean difference is significant at the .05 level.

Table 5J(2) : Testing the impact of oven type on the TFA content of smoked-dry Sardinella sp. stored fore the same duration (smoked fish dry matter content basis).

Storage month	Ovens		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Month 0	FTT	Chorkor smoker	-1.033*	,405	,016	-1,859	-,206
		Metal Drum	-4.643*	,384	,000	-5,427	-3,859
	Chorkor smoker	FTT	1.033*	,405	,016	,206	1,859
		Metal Drum	-3.611*	,384	,000	-4,395	-2,826
	Metal Drum	FTT	4.643*	,384	,000	3,859	5,427
		Chorkor smoker	3.611*	,384	,000	2,826	4,395
Month 3	FTT	Chorkor smoker	-.760*	,362	,044	-1,499	-,021
		Metal Drum	-3.696*	,362	,000	-4,435	-2,957
	Chorkor smoker	FTT	.760*	,362	,044	,021	1,499
		Metal Drum	-2.936*	,362	,000	-3,675	-2,197
	Metal Drum	FTT	3.696*	,362	,000	2,957	4,435
		Chorkor smoker	2.936*	,362	,000	2,197	3,675
Month 6	FTT	Chorkor smoker	-,715	,405	,088	-1,541	,111
		Metal Drum	-3.548*	,405	,000	-4,374	-2,721
	Chorkor smoker	FTT	,715	,405	,088	-,111	1,541
		Metal Drum	-2.833*	,405	,000	-3,659	-2,006
	Metal Drum	FTT	3.548*	,405	,000	2,721	4,374
		Chorkor smoker	2.833*	,405	,000	2,006	3,659

*. The mean difference is significant at the .05 level.

REFERENCES

Aboud, F.E., and Singla, D.R. (2012). Challenges to changing health behaviours in developing countries: a critical review. Social Science and Medicine 75: 589-594. http://dx.doi.org/10.1016/j.socscimed.2012.04.009

Adeyeye, S. A. O., and Oyewole, O. B. (2016). An overview of traditional fish smoking in Africa. Journal of Culinary Science and Technology, 14:3, 198-215, http://dx.doi.org/10.1080/15428052.2015.1102785

Adu-Gyamfi, A. (2006). Studies on the microbiological quality of smoked fish in some markets in Accra, Ghana. Ghana Journal of Science 46, 67 – 75. http://dx.doi.org/10.4314/gjs.v46i1.15918

Aduomih, A.A.O. (2019). Gender analysis: Ghana's artisanal fisheries 2019. https://ejfoundation.org/reports/gender-analysis-ghanas-artisanal-fisheries-2019 Accessed 22nd June 2020

Ahadzi, D. (2017). Personal communication. Fish processor with over three decades of fish smoking and trading experience in Ghana.

Akinwumi, F.O., and Adegbehingbe, K.T. (2015). Microbiological analysis of three smoked fish obtained from Ondo State, Nigeria. Food and Public Health 5(4):122-126. DOI: 10.5923/j.fph.20150504.04

Akpambang, V. O. E., Purcaro, G., Lajide, L., Amoo, I. A., Conte, L. S., and Moret, S. (2009). Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat. Food Additives and Contaminants: Part A, 26(7), 1096–1103. https://doi.org/10.1080/02652030902855406

American Oil Chemists Society (AOCS). (1990). Official Method Ce 1b-89 for marine oils. The American Oil Chemists' Society

Amuna, N.N. (2014). Food safety management practices in the traditional fish processing sector in Ghana and the microbiological safety of selected processed fish products from Ghana. Master Thesis. University of Greenwich, UK.

Andani, Z., Jaeger, S. R., Wakeling, I. N., and MacFie, H. J. H. (2001). Mealiness in apples: Towards a multilingual consumer vocabulary. Journal of Food Science, 66, 872–879. https://doi.org/10.1111/j.1365-2621.2001.tb15189.x

Anihouvi, D.G.H., Kpoclou, Y.E., Massih, M.A., Afe, O.H.I., Assogba, M.F., Covo, M., Scippo, M.L., Hounhouigan, D.J., Anihouvi, V., and Mahillon, J. (2018). Microbiological characteristics of smoked and smoked–dried fish processed in Benin. Food Science and Nutrition. DOI: 10.1002/fsn3.1030

Anoh, K.P., Ouattara, S., Ossey, Y.B., Dembélé, A.A., and Traoré, K.S. (2017). Santé des femmes transformatrices, sécurité sanitaire des produits et impact environnemental des systèmes de fumage de poisson dans les communautés de pêche artisanale – Étude pour des systèmes alimentaires durables. FAO Fisheries and Aquaculture Report / FAO Rapport sur les pêches et l'aquaculture No. 1228. Rome, FAO. 355 pp.

Ares, G., Jaeger, S.R., Bava, C.M., Cheang, S.K., Jin, D., Gimenez, A., Vidal, L., Fiszman, S.M., and Varela, P. (2015). CATA questions for sensory product characterization: Raising awareness of biases. Food Quality and Preference, 30:114 - 127. http://dx.doi.org/10.1016/j.foodqual.2013.04.012

Arias, C. (2009). Chilled and frozen raw fish. In Fernandes, R. (Ed). Microbiology handbook: fish and seafood. Letterhead Publishing, Surrey, UK. P4

Asafo, C.K. (1995). The fisheries sub-sector capacity building project. The Fisherman, Vol. 2 No.2. Newsletter of the Fisheries Department, Ministry of Food and Agriculture, Ghana.

Asiedu, B., Failler, P., and Beygens, Y. (2018). Ensuring food security: an analysis of the industrial smoking fishery sector of Ghana. Agric and Food Secur 7:18. https://doi.org/10.1186/s40066-018-0187-z

Assad, N.A., Balmes, J., Mehta, S., Cheema, U., and Sood, A. (2015). Chronic obstructive pulmonary disease secondary to household air pollution. Semin. Respir. Crit. Care Med. 36, 408–421. DOI: 10.1055/s-0035-1554846

Atta-Mills, J.E., Alder, J., and Sumaila, U.R. (2004). The decline of a regional fishing nation: The case of Ghana and West Africa. Natural Resources Forum 28 (2004) 13-21

Baidoo-Tsibu, G. (2016). Green means upholding the fisheries laws. http://www.MoFAD.gov.gh/green-means-upholding-the-fisheries-laws/ Accessed 22 September, 2016.

Bank of Ghana. (2008). The Fishing Sub-sector and Ghana's Economy. https://www.bog.gov.gh/privatecontent/Research/Sector%20Studies/fisheries_completerpdf.pdf Accessed on 20 March, 2015

Bansal, V., and Kim, K. H. (2015). Review of PAH contamination in food products and their health hazards. Environment International, 84, 26–38. https://doi.org/10.1016/j.envint.2015.06.016

Barlow, S., Renwick, A.G., Kleiner, J., Bridges, J.W., Busk, L., Dybing, E., Edler, L., Eisenbrand, G., Fink-Gremmels, J., Knaap, A., Kroes, R., Liem, D., Müller, D.J.G., Page, S., Rolland, V., Schlatter, J., Tritscher, A., Tueting, W., Würtzen, G. (2006). Risk assessment of substances that are both genotoxic and carcinogenic—report of an

International Conference organized by EFSA and WHO with support of ILSI Europe. Food Chem. Toxicol. 44, 1636–1650. https://doi.org/10.1016/j.fct.2006.06.020

Barlow, S., and Schlatter, J. (2010). Risk assessment of carcinogens in food. Toxicol Appl Pharm 243 (2010) 180–190. http://doi.org/10.1016/j.taap.2009.11.004

Barstow, M. 2018. *Pterocarpus erinaceus.* The IUCN Red List of Threatened Species. https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T62027797A62027800.en Accessed 12 May 2020.

Basak., S., Şengör, G.F., and Karakoç, F.T. (2010). The detection of potential carcinogenic PAH using HPLC procedure in different smoked fish, case study: Istanbul, Turkey. Turk. J. Fish. Aquat. Sci. 10: 351-355. http://doi.org/10.4194/trjfas.2010.0307

Becker, K., Southwick, K., Reardon, J., Berg, R., and MacCormack, J. N. (2001). Histamine poisoning associated with eating tuna burgers. Journal of the American Medical Association, 285(10), 1327-1330. DOI: 10.1001/jama.285.10.1327

Benford, D., Bolger, P.M., Carthew, P., Coulet, M., DiNovi, M., Leblanc, J.-C., Renwick, A.G., Setzer, R.W., Schlatter, J., Smith, B., Slob, W., Williams, G., and Wildemann, T. (2010). Application of the margin of exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. Food Chem. Toxicol. 48S1, S42–S48. http://doi.org/10.1016/j.fct.2009.11.003

Biji, K.B., Ravishankar, C.N., Venkateswarlu, R., Mohan, C.O., and Gopal, T.K.S. (2016). Biogenic amines in seafood: a review. J Food Sci Technol 53(5):2210-2218 DOI 10.1007/s13197-016-2224-x

Bilgin, S., Ulusayin, M., Izci, L., and Gunlu, A. (2008). The Determination of the shelf life and some nutritional components of gilthead seabream (*Sparus aurata*) after cold and hot smoking. Turkish Journal of Veterinary and Animal Sciences, 32, 49–56.

Bligh, E.G., and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37(8), 911-917. DOI: 10.1139/o59-099

Bomfeh, K. (2011). Risk assessment for traditionally processed fish from informal markets in Accra and Tema. Master of Philosophy in Food Science Thesis. Department of Nutrition and Food Science, University of Ghana.

Bomfeh, K., Jacxsens, L., Amoa-Awua, W.K., Tandoh, I., Afoakwa, E.O., Gamarro, E.G., Diei-Ouadi, Y., and De Meulenaer, B. (2019). Reducing polycyclic aromatic hydrocarbon contamination in smoked fish in the Global South: a case study of an improved kiln in Ghana. J. Sci Food Agric 99:5417 – 5423. DOI 10.1002/jsfa.9802

Bongoni, R. (2014). Consumers and vegetables: effects of domestic processing on sensory and health properties. PhD Dissertation, Wageningen University, Netherlands.

Brans, J.P., and Mareschal, B. (2005). Promethee Methods. In Greco, S., Ehrgott, M. and Figueira, J.R. (Eds). Multiple Criteria Decision Analysis: State of the Art Surves (pp. 163-186). DOI:10.1007/0-387-23081-5_5

Brown, M. (2002). Hazard identification. In Brown, M. and Stinger, M. (Ed). Microbiological risk assessment of food processing. CRC Press, Boca Raton.

Brownell, B. (1983). A practical guide to improved fish smoking in West Africa. UNICEF. Available at http://www.greenlight2015.org/chorkor/Brownell/Intro.htm Accessed 25 April, 2020

Bruce, N., Dherani, M., Liu, R., Hosgood, H.D., Sapkota, A., Smith, K.R., Straif, K., Lan, Q. and Pope, D. (2015). Does household use of biomass fuel cause lung cancer? A systematic review and evaluation of the evidence for the GBD 2010 study. Thorax 70, 433–441. DOI:10.1136/thoraxjnl-2014-206625

Bryhni, E.A., Byrne, D.V., Rødbotten, M., Claudi-Magnussen, C., Agerhem, H., Johansson, M., Lea, P. and Martens, M. (2002). Consumer perceptions of pork in Denmark, Norway and Sweden. Food Qual. Preference 13 (5), 257–266. DOI: 10.1016/S0950-3293(02)00021-6

Bunková, L., Bunka, F., Klcovska, P., Mrkvicka, V., Dolezalova, M. and Kracmar, S. (2010). Formation of biogenic amines by Gram-negative bacteria isolated from poultry skin. Food Chemistry 121:203-3016. DOI:10.1016/j.foodchem.2009.12.012

Byrne, D.V., Bredie, W.L.P., Mottram, D.S., Martens, M., (2002). Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. Meat Science 61, 127-139. https://doi.org/10.1016/S0309-1740(01)00171-1

Cameron-Smith, D., Albert, B.B. and Cutfield, S. (2015). Fishing for answers: is oxidation of fish oil supplements a problem? Journal of Nutritional Science, 4(36): 1-2. DOI:10.1017/jns.2015.26

Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Morkore, T., Thomassen, M., et al. (2001). Relation of smoking parameters to the yield colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). Food Research International, 34, 537-550. https://doi.org/10.1016/S0963-9969(01)00069-2

Cartus, A. and Schrenk, D. (2017). Current methods in risk assessment of genotoxic chemicals. Food and Chemical Toxicology 106:574-582 http://dx.doi.org/10.1016/j.fct.2016.09.012

Centers for Disease Control (USA). (2016). Campylobacter, Salmonella led bacterial foodborne illnesses in 2016. https://www.cdc.gov/media/releases/2017/p0420-campylobacter-salmonella.html Accessed 20 June 2020

Champmartin, C., Jeandel, F. and Monnier, H. (2017). Maintenance of low-pressure car- burising furnaces: A source of PAH exposure. Ann. Work Expo Health, 61(3), 321–332. https://doi.org/10.1093/annweh/wxw024

Chen, S.Y., Wang, L.Y., Lunn, R.M., Tsai, W.Y., Lee, P.H., Lee, C.S., Ahsan, H., Zhang, Y.J., Chen, C.J., and Santella, R.M. (2002) Polycyclic aromatic hydrocarbon–DNA adducts in liver tissues of hepatocellular carcinoma patients and controls. Int J Cancer 99:14–21

Chow, C.K. (2007). Fatty acids in foods and their health implications (3rd Ed.). Taylor and Francis.

Christie, W.W. (2018). Mass Spectrometry of Methyl Esters: Epoxy, Furanoid and Alkoxy Fatty Acids. Opgeroepen op april 21, 2020, van lipidhome.co.uk: https://www.lipidhome.co.uk/ms/methesters/me-epoxy/index.htm Accessed 28 November 2019

Ciecierska, M., and Obiedziński, M. (2007). Influence of Smoking Process on Polycyclic Aromatic Hydrocarbons ' Content. Acta Sci. Pol., Technol. Aliment., 6(4), 17–28.

Codex Alimentarius Commission (CAC). (1969). General principles of food hygiene. CAC/RCP 1-1969. http://www.fao.org/fao-who-codexalimentarius/codex-texts/codes-of-practice/en/ Accessed 18 May 2020

Codex Alimentarius Commission (CAC). (1999). Principles and guidelines for microbial risk assessment. CAC/GL 30. http://www.fao.org/3/y1579e/y1579e05.htm Accessed 14 July, 2019.

Codex Alimentarius Commission (CAC). (2001). Procedural Manual, 12th ed.,p. 175. Joint FAO/WHO Food Standards Programme, FAO, Rome, Italy.

Codex Alimentarius Commission (CAC). (2003). Code of practice for fish and fishery products. CAC/RCP 52-2003. http://www.fao.org/fao-who-codexalimentarius/codex-texts/codes-of-practice/en/ Accessed 18 May 2020

Codex Alimentarius Commission (CAC). (2009). Code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons from smoking and direct drying processes. CAC/RP 68-2009. Available at www.fao.org/input/download/standards/11257/CXP_068e.pdf Accessed 18 May 2020

Codex Alimentarius Commission (CAC). (2013). Standard for smoked fish, smoke-flavoured fish and smoke-dried fish. CODEX STAN 311–2013. Available at www.fao.org/input/download/standards/13292/CXS_311e.pdf Accessed 18 May 2020

Codex Alimentarius Commission (CAC). (2015). Procedural Manual, 24th Ed. Joint FAO/WHO Food Standards Programme, FAO, Rome, Italy. Available at http://www.fao.org/3/a-i5079e.pdf Accessed 20 September, 2020

Coffie, A. (2003). Processing Environment and the microbial quality of smoked fish in the Accra and Tema Municipalitiees. MPhil Thesis. Department of Environmental Science, University of Ghana.

Cropotova, J., Mozuraityte, R., Standal, I.B. and Rustad, T. (2019). Assessment of lipid oxidation in Atlantic mackerel (Scomber scombrus) T subjected to different antioxidant and sous-vide cooking treatments by conventional and fluorescence microscopy methods. Food Control 104: 1-8. DOI.org/10.1016/j.foodcont.2019.04.016

Dean A.G., Sullivan, K.M., and Soe, M.M. (2013). OpenEpi: Open source epidemiologic statistics for public health. https://www.openepi.com/Menu/OE_Menu.htm Accessed 8th November 2016

Dennis, S.B., Miliotis, M.D., and Buchanan, R.L. (2002). Hazard characterization/dose-response assessment. In Brown, M. and Stinger, M. (Ed). Microbiological risk assessment of food processing. CRC Press, Boca Raton.

Derelanko, M.J. and Hollinger, M.A. (2001). Handbook of toxicology, 2nd Ed. CRC Press.

Diei-Ouadi, Y. and Mgawe, Y. I. (2011). Post-harvest fish loss assessment in small-scale fisheries: a guide for the extension officer. FAO Fisheries and Aquaculture Technical Paper 559. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. http://www.fao.org/docrep/014/i2241e/i2241e.pdf Accessed 13 May 2020

Diggs, D.L., Huderson, A.C., Harris, K.L., Myers, J.N., Banks, L.D., Rekhadevi, P.V., Niaz, M.S., and Ramesh, A. (2011). Polycyclic aromatic hydrocarbons and digestive tract cancers: a perspective. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 29(4):324-57.
http://doi.org/10.1080/10590501.2011.629974

Duedahl-Olesen, L., Christensen, J.H., Højgård, A., Granby, K., and Timm-Heinrich, M. (2010). Influence of smoking parameters on the concentration of polycyclic aromatic hydrocarbons (PAHs) in Danish smoked fish. Food Addit Contam A, 27:9, 1294-1305 https://doi.org/10.1080/19440049.2010.487074

Essumang, D. K., Dodoo, D. K., and Adjei, J. K. (2012). Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish products. Journal of Food Composition and Analysis, 27(2), 128–138. https://doi.org/10.1016/j.jfca.2012.04.007

Essumang, D. K., Dodoo, D. K., and Adjei, J. K. (2013). Effect of smoke generation sources and smoke curing duration on the levels of polycyclic aromatic hydrocarbon (PAH) in different suites of fish. Food and Chemical Toxicology, 58, 86–94. https://doi.org/10.1016/j.fct.2013.04.014

Essumang, D. K., Dodoo, D. K., and Adjei, J. K. (2014). Effective reduction of PAH contamination in smoke cured fish products using charcoal filters in a modified traditional kiln. Food Control, 35(1), 85–93. https://doi.org/10.1016/j.foodcont.2013.06.045

European Commission (2005). Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foods. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073andfrom=EN Accessed 20 June 2020

European Commssion (2006). Commission Regulation (EC) No. 1881/2006. Setting Maximum Levels of Certain Contaminants in Foodstuffs. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF Accessed 20 June 2020

European Commission (2007). Commission Regulation (EC) No. 1441/2007. Amending Regulation (EC) No 2073/2005 on microbiological criteria for foods. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:322:0012:0029:EN:PDF Accessed 20 June 2020

European Commission (2011). Commission Regulation (EC) No. 835/2011. Amending Regulation (EC) No. 1881/2006 as regards maximum levels for certain polycyclic aromatic hydrocarbons in foodstuffs. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0004:0008:EN:PDF Accessed 20 June 2020

European Food Safety Authority (EFSA). (2005). Opinion of the scientific committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. The EFSA Journal (2005) 282, 1-31. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2005.282

European Food Safety Authority (EFSA). (2008). Polycyclic aromatic hydrocarbons in food. Opinion of the Panel on Contaminants in the Food Chain. EFSA Journal (2008) 724, 1-114 Available at http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/724.pdf Accessed 20 June 2020

European Food Safety Authority (EFSA). (2012). Risk communication. Available at https://www.moh.gov.cy/Moh/fsc/fsc.nsf/All/743202731E10D0F5C2257A9C0038E093/$file/riskcommguidelines170524.pdf

Ewa, B., and Danuta, M. S. (2017). Polycyclic aromatic hydrocarbons and PAH-related DNA adducts. Journal of Applied Genetics, 58(3), 321–330. DOI: 10.1007/s13353-016-0380-3

Eygue, M., Richard-Forget, F., Cappelier, J.M., Pinson-Gadais, L. and Membre, J.M. (2020). Development of a risk-ranking framework to evaluate simultaneously biological and chemical hazards related to food safety: Application to emerging dietary practices in France. Food Control 115: 107279. DOI.org/10.1016/j.foodcont.2020.107279

Fazil, A. Rajic, A., Sanchez, J., and McEwen, S. (2008). Choices, choices: the application of multi-criteria decision analysis to a food safety decision making problem. Journal of Food Protection, 2008; 71:2323–2333. DOI: 10.4315/0362-028x-71.11.2323

Ferrer-Gallego, R., Hernandez-Hierro, J.M., Rivas-Gonzalo, J.C. and Escribano-Bailon, M.T. (2014). Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: synergistic effect and modulation by aromas. Food Research International 63:1100-1107. DOI:10.1016/j.foodres.2014.05.049

Fotuhi, M., Mohassel, P. and Yaffe, K. (2009). Fish consumption, long-chain omega-3 fatty acids and risk of cognitive decline or Alzheimer disease: a complex association. Nature Clinical Practice Neurology 5(3):140-152. DOI: 10.1038/ncpneuro1044

Frankel, E.N. (2012). Lipid oxidation. Woodhead Publishing, Oxford.

Garre, A., Boue, G., Fernandez, P.S., Membre, J.M., and Egea, J.A. (2020). Evaluation of multicriteria decision analysis algorithms in food safety: a case study on emerging zoonoses prioritization. Risk Analysis, Vol. 40, No.2. DOI: 10.1111/risa.13391.

Gehle, K. (2009). Toxicity of polycyclic aromatic hydrocarbons. https://www.atsdr.cdc.gov/csem/pah/docs/pah.pdf Accessed 11 April 2019

Ghana Export Promotion Council (GEPC). (2010). The Export of Fish from Ghana. http://www.gepcghana.com/fish.php Accessed 5 January, 2015

Ghana Living Standards Survey. (2008). Report of the Fifth Round. http://www.statsghana.gov.gh/docfiles/glss5_report.pdf Accessed 5 January, 2015.

Ghana Standards Authority (GSA). (2014). Fisheries Product Regulation (Subsidiary Legislation).

Ghana Standards Authority (GSA). (2018). Introduction of revised fish standards. https://www.gsa.gov.gh/2018/05/ghana-standards-authority-introduces-revised-fish-standards/ Accessed on 18 April, 2020.
Ghana Statistical Service (GSS). (2012). Ghana 2010 population and housing census. https://www.statsghana.gov.gh/gssmain/storage/img/marqueeupdater/Census2010_Summary_report_of_final_results.pdf Accessed 5 January 2015

Girard, P. 2002). Charcoal production in Africa: what future? Unasulva 211, Vol. 53. http://www.fao.org/tempref/docrep/fao/005/y4450e/y4450e05.pdf Accessed on 10 June, 2020

Goicoechea, E. and Guillen, M.D. (2010). Analysis of hydroperoxides, aldehydes and epoxides by ^{1}H Nuclear Magnetic Resonance in sunflower oil oxidizedat 70 and 100 ^{o}C. Journal of Agricultural and Food Chemistry, 58(10), 6234-6245. https://doi.org/10.1021/jf1005337

Gordon, S.B., Bruce, N.G., Grigg, J., Hibberd, P.L., Kurmi, O.P., Lam, K.B., Mortimer, K., Asante, K.P., Balakrishnan, K., Balmes, J., Bar-Zeev, N., Bates, M.N., Breysse, P.N., Buist, S., Chen, Z., Havens, D., Jack, D., Jindal, S., Kan, H., Mehta, S., Moschovis, P., Naeher, L., Patel, A., Perez-Padilla, R., Pope, D., Rylance, J., Semple, S., and Martin., W.J. (2014). Respiratory risks from household air pollution in low- and middle-income countries. Lancet Respir. Med. 2, 823–860. DOI: 10.1016/S2213-2600(14)70168-7

Grace, D. (2015). Food safety in low- and middle-income countries. Int. J. Environ. Res. Public Health, 12, 10490-10507. DOI:10.3390/ijerph120910490

Greene, J.F., Newman, J.W., Williamson, K.C., and Hammock, B.D. (2000). Metabolism of monoepoxides of methyl linoleate: bioactivation and detoxification. Archives of Biochemistry and Biophysics, 376(2), 420-432
DOI: 10.1006/abbi.2000.1753

Gunstone, F.D., and Jacobsberg, F.R. (1972). Fatty acids, part 35: the preparation and properties of the complete series of methyl epoxyoctadecanotes. Chemistry and Physics of Lipids, 15(2), 174-188 https://doi.org/10.1016/0009-3084(72)90031-X

Hanlon, P., Brorby, G.P. and Krishan, M (2016). A Risk-Based Strategy for Evaluating Mitigation Options for Process-Formed Compounds in Food: Workshop Proceedings. International Journal of Toxicology Vol. 35(3) 358-370.
DOI: 10.1177/1091581816640262

Heggum, C. (2011). Risk analysis. Encyclopedia of Diary Sciences. 2nd Ed. https://doi.org/10.1016/B978-0-12-374407-4.00461-1

Heggum, C. (2014). Risk analysis and quantitative risk management. Encyclopedia of Meat Sciences (3), 226 – 234. https://doi.org/10.1016/B978-0-12-384731-7.00166-5

Howard, J. W., and Fazio, T. (2018). Polycyclic aromatic hydrocarbons in foods. In CRC Handbook of Naturally Occurring Food Toxicants (pp. 161–190). CRC Press. https://doi.org/10.1201/9781351072946

Hu, Y., Huang, Z., Li, J., and Yang, H. (2012). Concentrations of biogenic amines in fish, squid and octopus and their changes during storage. Food Chemistry 135, 2604 – 2611. DOI: 10.1016/j.foodchem.2012.06.121

Hungerford, J.M. (2010). Scrombroid poisoning: a review. Toxicon 56, 2, 231-243. https://doi.org/10.1016/j.toxicon.2010.02.006

Huss, H.H., Ababouch, L., and Gram, L. (2004). Assessment and management of seafood safety and quality. FAO Technical Series Paper 444. Food and Agriculture of the United Nations, Rome.

Ineyougha, E.R., Orutugu, L.A., and Izah, S.C. (2015). Assessment of microbial quality of smoked *Trachurus trachurus* sold in some markets of three South-south States, Nigeria. Int. J. Food Res. 2: 16-23.

Ingenbleek, L., Veryand, B., Adebgoye, A., Hossou, S.E., Kone, A.B., Oyedele, A.D., Kisito, C.S.K.J., Dembele, K.Y., Eyangoh, S., Verger, P., Leblanc, J.C., Duran, S., Venisseau, A., Merchad, P., and Le Bizec, B. (2019). Polycyclic aromatic hydrocarbons in foods from the first regional total diet study in Sub-Saharan Africa: contamination profile and occurrence data. Food Control 103: 133 - 144. DOI.org/10.1016/j.foodcont.2019.04.006

International Association for Research on Cancer. (2010) Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum 92:1–853

International Association for Research on Cancer. (2012). IARC Monographs 92, 100F https://publications.iarc.fr/123 Accessed 10 May 2020

International Association of Fish Inspectors. (2017). Statement from the International Association of Fish Inspectors on the safety of smoked fishery products. http://www.iafi.net/resources/Documents/IAFI%20Statement%20on%20PAH%20FinalEN.PDF Accessed 13 May 2019

Joint FAO/WHO. (1998). The application of risk communication to food standards and safety matters. FAO Food and Nutrition paper 70. Rome, Food and Agriculture Organization. ftp://ftp.fao.org/docrep/fao/005/x1271e/x1271e00.pdf

Joint FAO/WHO Expert Committee on Food Additives (JECFA). (2005). Evaluation of Certain Food Contaminants. 64th Report. WHO Technical Report Series 930. p.78.

Joint FAO/WHO Food Standards Programme. (2015). Procedural manual of the Codex Alimentatrius Commission. 24th Edition. Rome. http://www.fao.org/3/a-i5079e.pdf Accessed 20 October 2020

Kim, M. J., Hwang, H.H., and Shin, H.S. (2014). Evaluation of Polycyclic Aromatic Hydrocarbon Contents and Risk Assessment for Fish and Meat Products in Korea. Food Sci. Biotechnol. 23(3):991-998. DOI 10.1007/s10068-014-0134-0

Kim, M.K., Mah, J.H., and Hwang, H.J. (2009). Biogenic amine formation and bacterial contribution in fish, squid and shellfish. Food Chemistry 116: 87-96. DOI:10.1016/j.foodchem.2009.02.010

Kirezieva, K., Luning, P.A., Jacxsens, L., Allende, A., Johannessen, G.S., Tondo, E.C., Rajkovic, A., Uyttendaele, M., and Boekel, M.A.J.S (2015). Factors affecting the status of food safety management systems in the global fresh produce chain. Food control. 52, 85-97. DOI: 10.1016/j.foodres.2013.03.023

Komata, Y. (1990). Umami taste of seafoods. Food Rev. Int., 6(4): 457-487. https://doi.org/10.1080/87559129009540887

Komprda, T., Sladkova, P., and Dohnal, V. (2009). Biogenic amine content in dry fermented sausages as influenced by a producer, spice mix, starter culture, sausage diameter and time of ripening. Meat Science 83, 543-542. DOI:10.1016/j.meatsci.2009.07.002.

Kris-Etherton P.M., Harris, W.S., Appel, L.J. and American Heart Association Nutrition Committee. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106: 2747–57. https://doi.org/10.1161/01.CIR.0000038493.65177.94

Laky, S. (2019). Food safety fact sheet of the Europian Union. Available at https://www.europarl.europa.eu/factsheets/en/sheet/51/food-safety

Lammerding, A.M. and Fazil, A. (2000). Hazard identification and exposure assessment for microbial food safety risk assessment. International Journal of Food Microbiology 58, 147-157.

Lawless, H. T., and Heymann, H. (2010). Sensory evaluation of food: Principles and practice. 2nd Edition. Springer, New York.

Lee, Y., Lee, S-H. and Schmidt, S.J. (2008). Probing the sensory properties of food materials with nuclear magnetic resonance spectroscopy and imaging. In Webb, G.A. (Ed.). Modern magnetic resonance. Springer, IL. pp 1889-1894. DOI: 10.1007/1-4020-3910-7_215

Lerda, D. (2011). Polycyclic aromatic hydrocarbons (PAHs) factsheet. European Commission Joint Research Centre-Institute for Reference Materials and Measurements. JRC 66955-2011. Available at https://ec.europa.eu/jrc/sites/jrcsh/files/Factsheet%20PAH_0.pdf

Leuschner, R.G.K., Hristova, A., Robinson, T., and Hugas, M. (2013). The Rapid Alert System for Food and Feed (RASFF) database in support of risk analysis of biogenic amines in food. Journal of Food Composition and Analysis 29 (2013) 37-42. https://doi.org/10.1016/j.jfca.2012.09.004

Li, Z., Trinidad, D., Pittman, E. N., Riley, E. A., Sjodin, A., Dills, R. L., Paulsen M. and Simpson, C. D. (2016). Urinary polycyclic aromatic hydrocarbon metabolites as biomarkers to woodsmoke exposure-results from a controlled exposure study. J Expo Sci Env Epid, 26(3), 241–248. https://doi.org/10.1038/jes.2014.94

Lu, J.Y., Pace, R.D., and Plahar, W.D. (1991). Storage conditions and microbial quality of smoked dry herring in Ghana. Journal of Food Protection, Vol. 54, No. 7, 557-559. https://doi.org/10.4315/0362-028X-54.7.557

Mahugija, J. A. M. Njale, E. (2018). Effects of washing on the polycyclic aromatic hydrocarbons (PAHs) contents in smoked fish. Food Control, 93. 139-14 https://doi.org/10.1016/j.foodcont.2018.05.050

Maqsood, S., Benjakul, S., and Kamal-Eldin, A. (2012). Haemoglobin-mediated lipid oxidation in fish muscle: a review. Trens in Food Science and Technology 28: 33-43. http://dx.doi.org/10.1016/j.tifs.2012.06.009

Marc, C., Kaakey, R. and Mbofung, C.M.F. (1997). Effect of salting and smoking method on the stability of lipid and microbiological quality of Nile perch (*Lates niloticus*). Journal of Food Quality 22: 517-528 https://doi.org/10.1111/j.1745-4557.1998.tb00541.x

Metrowatch. 2015. Expert warns Nigerians against eating locally-smoked fish. In: Metrowatchonline.com [online]. http://metrowatchonline.com/expert-warns-nigerians-against-eating-locally-smoked-fish/ Accessed 18 June 2020

Michon, C., O'Sullivan, M.G., Delahunty, C.M., and Kerry, J.P. (2010). Study on the influence of age, gender and familiarity with the product on the acceptance of vegetable soups. Food Quality and Preference 21, 478-488. DOI: 10.1016/j.foodqual.2010.01.003

Mičulis, J., Valdovska, A., Šterna, V., and Zutis, J. (2011). Polycyclic aromatic hydrocarbons in smoked fish and meat, Agronomy Research 9 (Special Issue II), 439–442

Mindjimba, K. and Tiotsop, F. 2018. Factors determining FAO-Thiaroye processing technique adoption and sustainable use: evidence from selected sub-Saharan African countries. FAO Fisheries and Aquaculture Report No. 1228. Rome. www.fao.org/3/ca0374b/CA0374B.pdf Accessed 11 April 2020

Mindjimba, K., Rosenthal, I., Diei-Ouadi, Y., Bomfeh, K., and Randrianantoandro, A. (2019). FAO-Thiaroye processing technique: towards adopting improved fish smoking systems in the context of benefits, trade-offs and policy implications from selected developing countries. FAO Fisheries and Aquaculture Paper no. 634. Rome. FAO. 160 pp.

Ministry of Food and Aquaculture Development (MoFAD). (2017). Statistics and reports, fish production. https://www.mofad.gov.gh/publications/statistics-and-reports/ Accessed 8 April, 2018

Mohammadi, M., and Valizadeh-kakhki, F. (2018). Polycyclic aromatic hydrocarbons determination in grilled beef and chicken. Polycyclic Aromatic Compounds, 38(5), 434–444. https://doi.org/10.1080/10406638.2016.1236824

Moreno, R. B., and Torres, E. A. (2001). Histamine levels in fresh fish, a quality index. Institute of Food Technologists (IFT) Meeting, New Orleans, LA.

Mozuraityte, R., Kristinova, V., Standal, I.B., Carvajal, A.K. and Mariz, Aursand. (2016). Oxidative stability and shelf life of fish oil. In Hu, M. and Jacobsen, C.(Eds.). Oxidative stablity and shelf life of foods containing oils and fats. AOCS Press, Amsterdam. http://dx.doi.org/10.1016/B978-1-63067-056-6.00005-7

Mubiru, E., Shrestha, K., Papastergiadis, A., De Meulenaer, B. (2013). Improved gas chromatography-flame ionization detector analytical method for the analysis of epoxy fatty acids. Journal of chromatography A. 1318, 217-225. https://doi.org/10.1016/j.chroma.2013.10.025

Ndiaye, O. and Diei-Ouadi, Y. (2011). Smoking healthy and eating healthy fish: performance of the FAO-Thiaroye system, an improved design of kiln with particular focus on the control of polycyclic aromatic hydrocarbons (PAH). FAO Fisheries and Aquaculture Report No. 990. http://www.fao.org/docrep/017/i3093b/i3093b.pdf Accessed on 6 February, 2020

Ndiaye, O., Komivi, B.S. and Diei-Ouadi, Y. (2015). Guide for developing and using the FAO-Thiaroye Processing Technique. Food and Agriculture Organization of the United Nations. http://www.fao.org/3/a-i4174e.pdf Accessed on 14 September, 2016

Neely, G. and Borg, G. (1999). The perceived intensity of caffein aftertaste: tasters versus nontasters. Chemical Senses, 24(1):19-21. https://doi.org/10.1093/chemse/24.1.19

Nerquaye-Tetteh, G. (1999). The transfer, adoption and commercialisation of the Chorkor smoker: The success story of effective linkage practices by the Food Research Institute. https://csirspace.foodresearchgh.site/handle/123456789/1257 Accessed 20 June 2020

Nerquaye-Tetteh, G.A., Dassah, A.L. and Quashie-Sam, S.J. (2002). Effect of wood type on the quality of smoked fish – Chrysichthys auratus. Ghana Jnl Agric. Sci. 35, 95-101. http://212.47.242.17:8080/jspui/bitstream/123456789/320/1/GJAS_35_1_Nerquaye_Tetteh_et%20al.pdf Accessed on 14 June, 2020

Newman, J.W., Watanabe, T. and Hammock, B.D. (2002). The simultaneous quantification of cytochrome P450 dependent linoleate and arachidonate metabolites in urine by HPLC-MS/MS. J. Lipid. Res. 43: 1563–1578. DOI 10.1194/jlr.D200018-JLR200

Ninomiya, K. (2002). Umami: a universal taste. Food Rev. Int., 18(1): 23-38. https://doi.org/10.1081/FRI-120003415

Njinkoue , J.M., Barnathan, G., Miralles, J., Gaydou, E.M., and Samb, A. (2002). Lipids and fatty acids in muscle, liver and skin of three edible fish from the Senegalese coast: *Sardinella maderensis*, *Sardinella aurita* and *Cephalopholis taeniops.* Comparative Biochemistry and Physiology Part B 131, 395-402. https://doi.org/10.1016/S1096-4959(01)00506-1

Nketsia-Tabiri, J. and Sefa-Dedeh, S. (2000). Quality attributes and utilization of cured fish products in Ghana. Journal of Applied Science and Technology (JAST) Vol.5, Nos. 1 and 2, 2000, pp. 148-155. https://www.ajol.info/index.php/jast/article/view/17403/0 Accessed on 12 July 2017

Nti, C. A., Plahar, W. A., and Larweh, P. M. (2002). Impact of adoption in Ghana of an improved fish processing technology on household income, health and nutrition. International Journal of Consumer Studies, 26(2), 102–108. https://doi.org/10.1046/j.1470-6431.2002.00210.x

Nyamekye, S.K. (2000). Microbiology of smoked fish sold on the Ghanaian market. Higher Diploma in Microbiological Techniques Dissertation. Institute of Science and Technology, London

O'Brien, J., Renwick, A.G., Constable, A., Dybing, E., Müller, D.J.G., Schlatter, J., Slob, W., Tueting, W., van Benthem, J., Williams, G.M., and Wolfreys, A. (2006). Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. Food Chem. Toxicol. 44, 1613–1635. http://doi.org/10.1016/j.fct.2006.07.004

O'Sullivan, M.G., Kerry, J.P. and Byrne, D.V. (2011). Use of sensory science as a practical commercial tool in the development of consumer-led processed meat products. In: Kerry, J.P. and Kerry, J.F. (Eds.), Processed Meats. Woodhead Publishing Ltd., United Kingdom. DOI: 10.1533/9780857092946.1.156

Ofori, P.O. 2017. Chorkor Smoker fish can cause cancer – Researchers recommend new technology. In: Graphic Online [online]. [Cited 21 September 2018]. www.graphic.com.gh/news/general-news/chorkor-smoker-fish-can-cause-cancer-researchers-recommend- new-technology.html Accessed on 7 November, 2017

Olabemiwo, O. M., Alade, A. O., Tella, A. C., and Adediran, G. O. (2011). Assessment of polycyclic aromatic hydrocarbons content in smoked C. gariepinnus and T. guineensis fish species available in Western Nigeria. Int. J. Basic Appl. Sci, 11(02), 135–150. https://doi.org/10.1080/10406638.2019.1710857

Olokor, J.O. (2003). Cost of fuelwood for fish smoking around Kanji Lake and economic prospects of the kanji solar tent fish dryer. Proceedings of fisheries society of Nigeria (FISON) .pp 105-109.

Oppey, D. (2002). Determination of microbial hazards and critical control points of handling and processing of smoked mackerel (Scomber japonicus). Thesis. Department of Nutrition and Food Science, University of Ghana.

Orellana-Coca, C., Aldercreutz, D., andersson, M.M., Mattiasson, B.., and Hatti-Kaul, R. (2005). Analysis of fatty acid epoxidation by high performance liquid chromatography coupled with evaporative light scattering detection and mass spectrometry. Chemistry and Physics of Lipdis 135, 189 - 199. DOI:10.1016/j.chemphyslip.2005.02.014

Ortiz, L. T., Rebolé, A., Velasco, S., Rodríguez, M. L., Treviño, J., Tejedor, J. L., et al. (2013). Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition, 19, 475–482.

Osaili, T.M., Griffis, C.L., Martin, E.M., Beard, B.L., Keener, A.E., and Marcy, J.A. (2007). Thermal inactivation of Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes in breaded pork patties. J Food Sci. 72(2):M56-M61. DOI:10.1111/j.1750-3841.2006.00264.x

Oterhals, A. and Vogt, G. (2013). Impact of extraction, refining and concentration stages on the stability of fish oil. In Jacobsen, C., Nielson, N.S., Horn, A.F. and Sorenson, A.D.M. (Eds). Food enrichment with omega-3 fatty acids. Woodhead Publishing. Oxford.

Overa, R. (2002), When sisters become competitors: Coastal women's access and utilisation of trawlers by-catch in Ghana. In: Neis, B., Binkley, M., Gerrard, S. and Maneschy, C. (Eds.) Gender, Globalization and Fisheries. Fernwood Publishing, Halifax, Canada.

Palm, L. M. N., Carboo, D., yeboah, P. P., Quasie, W. J., Gorleku, M. A., and Darko, A. (2011). Characterization of polycyclic aromatic hydrocarbons (PAHs) present in smoked fish from Ghana. Advanced Journal of Food Science and Technology, 3(5), 332–338.

Park, J. S., Lee, C. H., Kwon, E. Y., Lee, H. J., Kim, J. Y., and Kim, S. H. (2010). Monitoring the contents of biogenic amines in fish and fish products consumed in Korea. Food Control, 21, 1219-1226. https://doi.org/10.1016/j.foodcont.2010.02.001

Pecore, S., and Kellen, L. (2002). A consumer-focused QC/sensory program in the food industry. Food Quality and Preference 13, 369-374. https://doi.org/10.1016/S0950-3293(02)00058-7

Peñarubia, O.R., Randrianatoandro, A., and Diei-Ouadi, Y. (2017). Background paper for the discussion on benchmarking of fish smoking systems in Africa: advances in fish fish smoking and perspectives for a sustainable food system. Report and papers presented at the fourth meeting of professionals/experts in support of fish safety, technology and marketing in Africa. Elmina, Ghana. FAO Fisheries and Aquaculture Report No. 1228. FAO, Rome.

Phillips, D.H., 1999. Polycyclic aromatic hydrocarbons in the diet. Mutation Research 443 (1–2), 139–147. https://doi.org/10.1016/S1383-5742(99)00016-2

Quansah, R., Semple, S., Ochieng, C.A., Juvekar, S., Armah, F.A., Luginaah, I., and Emina, J. (2017). Effectiveness of interventions to reduce household air pollution and/or improve health in homes using solid fuel in low-and-middle income countries: A systematic review and meta-analysis. Environment International 103, 73-90. http://dx.doi.org/10.1016/j.envint.2017.03.010

Randrianantoandro, A. and Diei-Ouadi, Y. 2015. Climate-smart agriculture for fisheries: the FAO-Thiaroye fish processing technique. In: World Bank, FAO and IFAD. Gender in Climate-Smart Agriculture. Module 18 for the Gender in Agriculture Sourcebook. World Bank, FAO and IFAD. 84 pp. www.fao.org/3/a-i5546e.pdf. Accessed 14 August, 2018

Ravindra, K., Sokhi, R., and Van Grieken, R. (2008). Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. Atmospheric Environment, 42(13), 2895–2921. https://doi.org/10.1016/j.atmosenv.2007.12.010

Rebolé, A., Velasco, S., Rodríguez, M. L., Treviño, Alzueta, C., J., Tejedor, J. L. and Ortiz, L.T. (2015). Nutrient composition in the muscle and skin of fillets from farmed rainbow trout (Oncorhynchus mykiss). Food Chemistry 174, 614-620. http://dx.doi.org/10.1016/j.foodchem.2014.11.072

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., and Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific Journal of Tropical Biomedicine, 5(3), 182–189. https://doi.org/10.1016/S2221-1691(15)30003-4

Roesel, K. and Grace, D. (Eds). (2015). Food safety and informal markets: animal products in sub-Saharan Africa. Routledge. https://cgspace.cgiar.org/handle/10568/42438 Accessed 7 May, 2017

Ruiz-Capillas, C. and Moral, A. (2004). Free amino acids and biogenic amines in red and white muscle of tuna stored in controlled atmospheres. Amino Acids 26, 125–132. https://doi.org/10.1007/s00726-003-0054-4

Ruiz-Capillas, C. and Herrero, A.M. (2019). Impact of biogenic amines on food quality and safety. Foods, 8, 62. DOI:10.3390/foods8020062
Ruzante, J.M., Davidson, V.J., Caswell, J., Fazil, A., Cranfield, J.A.L., Henson, S.J., Anders, S.M., Schmidt, C., and Farber, J.M. (2010). A multifactorial risk prioritization framework for foodborne pathogens. Risk Analysis, Vol. 30, No. 5.
DOI: 10.1111/j.1539-6924.2009.01278.x

Sarower, M.H., Hasanuzzaman, A.F.M., Biswas, B., and Abe, H. (2012). Taste producing components in fish and fisheries products: A review. Intl. J. of Food Ferment. Technol 2(2):113-121.

Schaich, K.M. (2005). Lipid oxidation: Theoritical aspects. In Shahidi, F. (Ed). Bailey's industrial oil and fat products, 6th Edition. John Wiley and Sons, Inc., New Jersey, USA.

Scientific Committee on Food (SCF). (2002). Opinion of the Scientific Committee on Food on the risks to human health of polycyclic aromatic hydrocarbons in food expressed on 4th December 2002. Brussels, European Commission. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out153_en.pdf Accessed on 17 January, 2017

Secci, G., Parisi, G., Dasilva, G., Medina, I. (2016). Stress during slaughter increases lipid metabolites and decreases oxidative stability of farmed rainbow trout (*Oncorhynchus mykiss*) during frozen storage. Food Chem. 190, 5–11.
DOI: 10.1016/j.foodchem.2015.05.051

Sefa-Dedeh, S. (1993). Traditional Food Technology. In Macrae, R., Robinson, R., and Sadler, M.(eds). Encyclopaedia of Food Science, Food Technology and Nutrition. Academic Press, New York. pp 4600-4606.

Shallaby, A.R. (1997). Significance of biogenic amines to food safety and human health. Food Research Journal, Vol. 29. No. 7, pp. 675-690

Silla Santos, M. H. (1996). Biogenic amines: their importance in foods. International Journal of Food Microbiology, 29, 213.

Silva, B. O., Adetunde, O.T., Oluseyi, T.O., Olayinka, K.O., and Alo, B.I. (2011). Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. Afr. J. Food Sci. 5(7), pp. 384-391 https://academicjournals.org/journal/AJFS/article-full-text/FBD534550813

Šimko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 770(1–2), 3–18. https://doi.org/10.1016/S0378-4347(01)00438-8

Singh, L., Varshney, J. G., and Agarwal, T. (2016). Polycyclic aromatic hydrocarbons' formation and occurrence in processed food. Food Chemistry. 199 (2016) 768–781 https://doi.org/10.1016/j.foodchem.2015.12.074

SNV Netherlands Development Organisation, Central and Western Region Fishmongers Improvement Association, and Coastal Resources Center. (2016). Fishing community livelihood value chain development and post-harvest improvements: an extension strategy for the scale-up of improved smoker technologies. http://www.crc.uri.edu/download/GH2014_ACT086_CRC__CEW_SNV_FIN508.pdf Accessed 18 March, 2018

Srogi, K. (2007) Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ Chem Lett 5:169–196

Stołyhwo, A., and Sikorski, Z. E. (2005). Polycyclic aromatic hydrocarbons in smoked fish - A critical review. Food Chemistry, 91(2), 303–311. https://doi.org/10.1016/j.foodchem.2004.06.012

Stone, H., and Sidel, J. L. (2003). Sensory evaluation: Descriptive analysis. In Caballero, B. (Ed.) Encyclopedia of Food Sciences and Nutrition, 2nd Ed. Academic Press. https://doi.org/10.1016/B0-12-227055-X/01065-8

Stone, H., Belibaum, R.N., and Thomas, H.A. (2012). The organisation and operation of a sensory evaluation programme. Sensory evaluation Practices, 4th Edition. DOI: http://dx.doi.org/10.1016/B978-0-12-382086-0.00002-9

Stoud, G.D. 2001. Rigor in Fish - The effect on quality. http://www.fao.org/3/x5914e/x5914e00.htm#Contents

The Food and griculture Organization of the United Nations (FAO). (2005). Food Safety Risk Analysis. Part 1: An overview and framework manual. https://www.fsc.go.jp/sonota/foodsafety_riskanalysis.pdf

The Food and Agriculture Organization of the United Nations (FAO). (2007). Report and papers presented at the FAO Workshop on Fish Technology, Utilization and Quality Assurance. Bagamoyo, United Republic of Tanzania, 14–18 November 2005. Rapport et documents présentés à l'Atelier de la FAO sur la technologie, l'utilisation et l'assurance de qualité du poisson. Bagamoyo, République-Unie de Tanzanie, 14-18 novembre 2005. FAO Fisheries Report/FAO Rapport sur les pêches. No. 819. Rome, FAO. 262p.

The Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO). (2009). Principles and methods for the risk assessment of chemicals in food: dietary exposure assessment of chemicals in food. WHO, Geneva. https://www.who.int/publications/i/item/principles-and-methods-for-the-risk-assessment-of-chemicals-in-food Accessed 7 April, 2020

The Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO). (2013). Public health risks of histamine and other biogenic amines from Fish and Fishery Products. Meeting report. http://www.fao.org/3/a-i3390e.pdf Accessed 4 January, 2016

The Food and Agriculture Organization of the United Nations (FAO). (2014). Overview, parts, use and maintenance of FTT Thiaroye. Three-part videos on FTT Thiaroye available at the following links (accessed on :
https://www.youtube.com/watch?v=4ehj-lNscb8
https://www.youtube.com/watch?v=nmLak9hDLXM
https://www.youtube.com/watch?v=nk7eaZWTifs

The Food and Agriculture Organization of the United Nations (FAO). (2016). The state of world fisheries and aquaculture: contributing to food security and nutrition for all. Available at http://www.fao.org/3/a-i5555e.pdf

The Food and Agriculture Organization of the United Nations (FAO). (2017). Food safety risk management: evidence-informed policies and decisions, considering multiple factors. FAO, Rome.

The Food and Agriculture Organization of the United Nations (FAO). (2020). FAO guide to ranking food safety risks at the national level. Food Safety and Quality Series No 10. Rome. https://doi.org/10.4060/cb0887en

The United Nations (UN). (2015). Sustainable development goals. https://www.un.org/sustainabledevelopment/sustainable-development-goals/ Accessed 12 April, 2016

Towers, L. (2014). A guide to smoking for fish preservation. https://thefishsite.com/articles/a-guide-to-smoking-for-fish-preservation Accessed 10 June 2020

Tran, N., Miller, A.J. and Buchanan, N. (2006). Exposure assessment of foodborne pathogens. In Morris, P. (Ed). Food consumption and disease risk. CRC Press, Boca Raton.

Unnevehr, L. (2014). Food safety in developing countries: moving beyond exports. Global Food Security, 4, 24-29. http://dx.doi.org/10.1016/j.gfs.2014.12.001

Uyttendale, M., De Loy-Henderickx, A., Vermeulen, A., Jacxsens, L., Debevere, J., and Devlieghere, F. (2018). Microbiological Guidelines: support for interpretation of microbiological test results of foods. Die Keure Professional Publishing, Brugge, Belgium.

Van der Fels-Klerx,H.J., Van Asselt, E.D, Raley, M. Poulsen, M., Korsgaard, H., Bredsdorff, L., Nauta, M., D'agostino, M.,Coles, D., Marvin, H. J. P., and Frewer, L.J. (2018). Critical review of methods for risk ranking of food-related hazards, based on risks for human health. Critical Reviews in Food Science and Nutrition, 58:2, 178-193, DOI: 10.1080/10408398.2016.1141165

Vandemoortele, A. (2020). Reactivity of secondary lipid oxidation products in food systems. PhD Dissertation, Faculty of Bioscience Engineering, Ghent University, Belgium.

Van Kreijl, C. F., Knaap, A. G. A. C. and Van Raiij, J. M. A. (2006). Our Food, our Health - Healthy Diet and Safe Foods in the Netherlands (in Dutch), p. 364. Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands. Report 270555009.

Varlet, V., Serot, T., Monteau, F., Le Bizec, B., and Prost, C. (2007). Determination of PAH profiles by GC-MS/MS in salmon processed by four cold-smoking techniques. Food Additives and Contaminants 24(7): 744-757. DOI: 10.1080/02652030601139946

Vinci, G. and Antonelli, M.L. (2002). Biogenic amines: quality index of freshness in red and white meat. Food Control 13, 519-524. DOI: 10.1016/S0956-7135(02)00031-2

Vose, D. (2008). Risk analysis: a quantitative guide. John Wiley and Sons, West Essex, England

VP Solutions.(2013). Visual PROMETHEE 1.4 Manual. http://www.promethee-gaia.net/assets/vpmanual.pdf Accessed 23 June 2020.

Walpole, S.C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., and Roberts, I. (2012). The weights of nations: an estimation of adult human biomass. BMC Public Health 2012, 12:439. DOI: 10.1186/1471-2458-12-439

Ward, A. (2003). A study of the trade in smoked-dried fish from West Africa to the United Kingdom. FAO Fisheries Circular. No. 981. Rome, FAO. Abailable at http://www.fao.org/docrep/006/Y4530E/Y4530E00.HTM

Williams, P.L., James, R.C., and Roberts, S.M. (2000). Principles of toxicology: environmental and industrial application. 2nd Ed. John Wiley & Sons, New York.

World Health Organization. (2015). Estimates of the global burden of foodborne diseases. https://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/ Accessed 15 October, 2017

Yebra-Pimentel, I., Fernández-González, R., Martínez-Carballo, E., and Simal-Gándara, J. (2015). A critical review about the health risk assessment of pahs and their metabolites in foods. Crit Rev Food Sci Nutr. 55:10, 1383-1405 https://doi.org/10.1080/10408398.2012.697497

Yusop, S.,M., O'Sullivan, M.G., Kerry, J.F., Kerry, J.P., (2009). Sensory evaluation of Indian-style marinated chicken by Malaysian and European naïve assessors. Journal of Sensory Studies 24, 269-289. DOI: 10.1111/j.1745-459X.2009.00210.x

Zamora, R. and Hidalgo, F. (2016). The triple defensive barrier of phenolic compounds against the lipid oxidation-induced damage in food products. Trends in Food Science and Technology 54: 165-174. DOI: 10.1016/j.tifs.2016.06.006

Zelasney, J., Ford, A., Westlund, L., Ward, A. and Riego Peñarubia, O. (Eds). 2020. Securing sustainable small-scale fisheries: Showcasing applied practices in value chains, post-harvest operations and trade. FAO Fisheries and Aquaculture Technical Paper No. 652. Rome, FAO. https://doi.org/10.4060/ca8402en

Zelinkova, Z., and Wenzl, T. (2015). The Occurrence of 16 EPA PAHs in Food – A Review, Polycyclic Aromatic Compounds, 35:2-4, 248-284 http://doi.org/10.1080/10406638.2014.918550

Zhai, H., Yang, X., Li, L., Xia, G., Cen, J., Huang, H. and Hao, S. (2012). Biogenic amines in commercial fish and fish products sold in southern China. Food Control 25 (2012) 303 – 308 https://doi.org/10.1016/j.foodcont.2011.10.057

Zwietering, M.H., Jacxsens, L., Membré, J-M., Nauta, M and Peterz, M. (2016). Relevance of microbial finished product testing in food safety management. , Food Control. 60. p.31-43

CURRICULUM VITAE

CURRICULUM VITAE

Kennedy Bomfeh was born on 6th June, 1985 in Atebubu, Ghana, West Africa. He holds a Bachelor of Science Degree in Nutrition and Food Science and a Master of Philosophy Degree in Food Science, both from the University of Ghana. He has a number of certificates in food safety, including Sanitary/Phytosanitary (SPS) and Advanced Level Risk Analysis from the United States Department of Agriculture and the Tuskegee University (USA), HACCP Implementation from the Industry Council for Development (UK), Food Hygiene Inspection from the EU Better Training for Safer Food Africa Project, Food Safety and Quality Management Systems for fresh produce from Hassan II Institute of Agronomy and Veterinary Medicine (Morocco), and Food Safety, Quality Assurance Systems and Risk Analysis from Ghent University. He also has a certificate in Humanitarian Food Science and Technology, jointly issued by Ghent University and Polytech Lille (France).

Kennedy has a decade of work experience spanning areas such as teaching, quality assurance in industry and international consultancy in food safety. He was part of a team of experts that conduted a rapid appraisal of food safety risks in the farmed fish value chain in Egypt for WorldFish and the International Lifestock Research Institute, and has also understaken several field and desk assignments for the Fisheries and Aquaculture Department of the Food and Agriculture Organization of the United Nations.

He managed the production and distribution of a food supplement for addressing infant malnutrition in Ghana in a tripartite study among the Ajinomoto Co. Inc., (Japan), the Nevin Scrimshaw International Nutrition Foundation (USA) and the University of Ghana and subsequently served as a consultant for the commercial production of the supplement. At the maiden international food safety conference jointly organized by the FAO, the World Health Organization (WHO) and the African Union (AU), he presented a high-level background paper on policy considerations for the adoption of technologies for local food value chains in low and middle income countries.

Kennedy joined the Department of Food Technology, Safety and Health of the Faculty of Bioscience Engineering, Ghent University, in 2015 for his PhD. His study assessed the impacts of traditional and improved fish smoking techniques on the safety of smoked fish, to provide scientific evidence for food safety policy development vis-à-vis smoked fishery products in the Ghanaian context. He supervised a master dissertation during this period.

PUBLICATIONS IN A1 PEER-REVIEWED JOURNAL

Bomfeh, K., Jacxsens, L., Amoa-Awua, W. K., Tandoh, I., Afoakwa, E. O., Gamarro, E. G., Diei-Ouadi, Y., De Meulenaer, B. (2019). Reducing polycyclic aromatic hydrocarbon contamination in smoked fish in the Global South: a case study of an improved kiln in Ghana. Journal of the Science of Food and Agriculture 99 (12), 5417-5423. DOI 10.1002/jsfa.9802

Bomfeh, K., Jacxsens, L., Amoa-Awua, W.K., GarridoGamarro, E., Diei-Ouadi, Y., De Meulenaer, B. Risk assessment of polycyclic aromatic hydrocarbons (PAHs) in smoked *Sardinella* sp. in Ghana: impact of a kiln intervention on public health protection. *Risk Analysis* (Under Review)

TECHNICAL PUBLICATIONS [POLICY SUPPORT DOCUMENTS]

Bomfeh, K., Jacxsens, L., De Meulenaer, B., Amoa-Awua, W.K. and Afoakwa, E.O. (2019). Policy recommendations and smallholder guidelines for improved fish smoking systems. FAO Fisheries and Aquaculture Circular No. 1178. Rome. FAO. 28 pp

Mindjimba, K., Rosenthal, I., Diei-Ouadi, Y., Bomfeh, K. and Randrianantoandro, A. (2019). FAO-Thiaroye processing technique: towards adopting improved fish smoking systems in the context of benefits, trade-offs and policy implications from selected developing countries. FAO Fisheries and Aquaculture Paper no. 634. Rome. FAO. 160 pp.

ORAL PRESENTATIONS

Bomfeh, K. (2019). Policy considerations for the development and adoption of technologies for local food value chains. The First FAO/WHO/AU International Food Safety Conference. 12-13 February 2019. African Union Headquarters, Addis Ababa, Ethiopia.

Bomfeh, K., Jacxsens, L., De Meulenaer, B., Amoa-Awua, W.K. (2019). From traditional to novel Kilns: risk-based improvement of the safety of smoked fish in Ghana. World Seafood Congress. 12th September, 2017. Reykjavik, Iceland

Bomfeh, K., Jacxsens, L., De Meulenaer, B., Amoa-Awua, W.K. (2019). Improving the safety of smoked fish through kiln design: The case of FAO-Thiaroye Technique in Ghana. African Network of Fish Technology and Safety Experts Meeting. 15th November, 2017. Elmina, Ghana.

POSTER PRESENTATIONS

Bomfeh, K., Jacxsens, L., De Meulenaer, B., Amoa-Awua, W.K. (2019). Impact of a kiln intervention on human exposure to polycyclic aromatic hydrocarbons (PAHs) in smoked fish in Ghana. International Association of Food Protection Annual Meeting. 22nd July, 2019. Louisville, USA..

Bomfeh, K., Jacxsens, L., De Meulenaer, B., Amoa-Awua, W.K. (2020). Hazard ranking in smoked fish in Ghana. International Association of Food Protection Annual Meeting. Cleveland, Ohia, USA. 22 – 25 October, 2020.

SUPERVISED DISSERTATION AS A TUTOR

De Henau, N. (2020). Gebruik van epoxyvetzuren als indicator voor oxidative stabiliteit van gerookte vis. Master dissertation, Ghent University.

PROFESSIONAL MEMBERSHIPS

International Association of Food Protection

Institue of Food Technololgists

Ghana Science Association