RADIATION PRESERVATION OF SMOKED GUINEA FOWL (*Numida meleagris)* MEAT FOR ENHANCED SHELF LIFE

BY

EVELYN AMA OTOO

(10601283)

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DECLARATION

I, Evelyn Ama Otoo, the author of this thesis hereby declare that except for references which have been duly cited, this work is the original research undertaken by me in the Department of Nuclear Agriculture and Radiation Processing of the School of Nuclear and Allied Sciences, University of Ghana, Legon under the supervision of Dr. Fidelis C. K. Ocloo and Prof. Victoria Appiah. This thesis has not been presented or published either in whole or in part for any other degree in this University or elsewhere.

Signature ……..…… Date……....

Evelyn Ama Otoo

(Student)

Signature …… Date……..

Dr. Fidelis C. K. Ocloo

(Principal Supervisor)

Signature …… Date……..

Prof. (Mrs.) Victoria Appiah

(Co-supervisor)

DEDICATION

This thesis is dedicated to the entire Otoo family especially my father, Mr. Elvis E. Otoo and grandfather, Mr. Frederick N. K. Otoo for their immense investment and support throughout my entire education.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemists
AOPs	-	Advanced Oxidation Processes
BNARI	-	Biotechnology and Nuclear Agriculture Research Institute
DDI	-	Dietary Daily Intake
EFSA	-	European Food Safety Authority
FAO	-	Food and Agriculture Organisation
GAEC	-	Ghana Atomic Energy Commission
GC/MS	-	Gas Chromatography/Mass Spectrometer
GHS	-	Ghana Health Service
GIF	-	Gamma Irradiation Facility
GSA	-	Ghana Standards Authority
HMW	-	High Molecular Weight
IACR	-	International Agency on Cancer Research
IAEA	-	International Atomic Energy Agency
LIPRC	-	Livestock Production and Research Centre
LMW	-	Low Molecular Weight
MALDI-TOF	-	Matrix-assisted Laser Desorption/Ionisation – Time-of-flight
NMIMR	-	Nouguchi Memorial Institute of Medical Research

NNRI	-	National Nuclear Research Institute
PAHs	-	Polycyclic Aromatic Hydrocarbons
RAMSRI	-	Radiological and Medical Sciences Research Institute
RTC	-	Radiation Technology Centre
SPE	-	Solid Phase Extraction
TEFs	-	Toxic Equivalent Factors
TEQs	-	Total Toxic Equivalents
USEPA	-	United States Environmental Protection Agency
WHO	-	World Health Organisation
XRF	-	X-ray Fluorescence

ABSTRACT

Guinea fowl (*Numida meleagris*) production is the main basis of livelihood to many Ghanaians with substantial role in nutrition and food security. Due to the nutritional quality of guinea fowl meat, it has resulted in processing of the meat into various shelf-stable and ready-to-eat meat products, such as smoked, grilled or fried meat. However, the current traditional guinea fowl meat processing methods do not always guarantee a prolonged shelf life and quality of the meat. Smoking also exposes the meat to potential health hazards associated with smoked foods mainly polycyclic aromatic hydrocarbons (PAHs) and their derivatives. The present study aimed at investigating the effect of gamma irradiation on the nutritional composition, smoke quality (PAHs) and shelf life of smoked guinea fowl meat stored at refrigerated temperature. Dressed guinea fowl meats were smoked, packaged and gamma irradiated with doses of 0, 2.5, 5.0 and 7.5 kGy at a dose rate of 0.74 kGy h^{-1}. Nutritional composition and PAHs concentrations were determined using standard analytical methods. Physicochemical, sensory and microbial properties of the treated meats were determined over refrigerated storage period using appropriate procedures. Gamma irradiation significantly ($p<0.05$) affected the major nutritional components of the meat. However, elemental (mineral) compositions of the meat were not significantly ($p>0.05$) affected. Sixteen (16) U.S. Environmental Protection Agency (USEPA) priority PAHs were detected in the smoked meat. Gamma irradiation significantly ($p<0.05$) reduced the PAHs concentrations and their carcinogenic derivatives drastically with undetectable levels of benzo[a]pyrene and other four high molecular weight (HMW) PAHs known to be carcinogenic to humans. The reduction of the PAHs concentrations were exponential with the

increasing irradiation doses. Titratable acidity (TA) and acid value (AV) decreased significantly ($p<0.05$) with gamma irradiation, but increased with storage. pH of the meat samples were however within the neutral range which was numerically insignificant to affect quality characteristics of the processed meat. Bacterial isolates identified on the basis of their mass spectra of protein profiles on the smoked guinea fowl meat were *Staphylococcus aureus*, *Serratia marcescens* and *Enterobacter cloacae*. The total bacterial counts decreased with increasing doses of gamma irradiation and storage. Irradiation had highly significant effects ($p<0.05$) on the reduction of microbial population. Irradiation had no significant effects ($p\geq0.05$) on the sensory attributes (aroma, colour, tenderness and taste) of the smoked guinea fowl meat; but the taste of the irradiated meat samples was influenced at the end of the seven weeks refrigerated storage period. Results obtained in the present study indicate that the successful utilization of gamma irradiation as a potent advanced oxidation process, will promote microbial decontamination, decomposition and elimination of harmful carcinogenic PAHs with minimal effect on nutritional and physicochemical properties, while enhancing the sensory and overall shelf life of smoked guinea fowl meat.

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Meat is known to be the skeletal muscle of ruminants, non-ruminants, and poultry (Papuc *et al.*, 2016). It is an essential source of proteins, vitamins and minerals required for proper growth and maintenance. Meat is known to be protein-rich and lipid-rich food that is highly perishable due to its high nutritional contents, chemical, microbiological and enzymatic processes occurring during processing/preservation, packaging, and storage (Adeyinka *et al.*, 2011). These properties may result in oxidative rancidity, discolouration, off-flavour, and sliminess, etc. (Adeyinka *et al.*, 2011). It is reported that the major source of these deteriorative changes are microorganisms, which renders the meat unacceptable and unsafe for consumption (Forrest *et al.*, 2001). Among the meats, poultry such as chicken and guinea fowl meats are much preferred as they are not associated with any cultural or religious taboos, and are highly perceived as healthy meat due to their high protein and low fat contents (Adeyinka *et al.*, 2007; Saina *et al.*, 2005; Guèye, 2000).

Guinea fowl meat is increasingly becoming an important poultry meat in the diet of many health conscious consumers, and as a key source of livelihood with substantial role in nutrition and food security (Issaka and Yeboah, 2016). Consumption of this white meat is gaining increase over red meat due to the high risk of cardiovascular diseases associated with red meat. In most cities of Ghana, it is a common sight to observe the sale of grilled and smoked guinea fowl at bars or clubs and along the roadside (Issaka and Yeboah, 2016). Because of its unique flavoursome

characteristics, it is mostly smoked (cured or uncured) or grilled with spices, and consumed without further processing. Alternatively, the meat may be purchased and stored for later consumption. Handling, transporting and storage of the meat may decrease its quality, due to cross contamination occurring along the food value chain. As a result, the microbial quality decrease substantially, and meats start producing off-flavour during storage which affect sensory qualities of the meat (Jongberg *et al.*, 2011; Zhou *et al.*, 2010; Lund *et al.*, 2007). The quality of fresh product, efficacy of processing operations, sanitation during handling and packaging, and maintaining adequate refrigeration (Selvan *et al.*, 2007) are some of the factors that affect the quality of the meat products consumed. When these factors are well controlled, foodborne illnesses will drastically reduce to its barest minimum, thus becoming safe for the public health.

Irradiation has become an effective means of processing and preservation of food products (Al-Bachir, 2016). Decontamination of food by ionizing radiation is known to be a safe, efficient, environmentally clean and energy efficient process (Farkas, 1998). Gamma irradiation is widely used for disinfestations and removal of some foodborne pathogens or/and microbial agents from foods, and has been proven to be more effective than traditional decontamination methods (Mansour and Al-Bachir, 1995). On the other hand, gamma irradiation has been considered an emerged technique for the reduction and elimination of other chemical contaminants such as the polycyclic aromatic hydrocarbons (PAHs) pollutants from the environment with a great application in industrial countries (Kim *et al.*, 2000). It is worth noting that there is inadequate information available on the effect of gamma irradiation on such contaminants in meat and poultry products although appreciable information on the effect of gamma irradiation on nutritional, microbiological and shelf life studies have

been reported (Hajare *et al.*, 2014; Farkas, 2006; Mahapatra *et al.*, 2005; ICGFI, 1999). Therefore, the present study seeks to utilize gamma irradiation as a decontamination technique to enhance the shelf life of smoked guinea fowl meat whilst maintaining microbiological, nutritional and chemical safety of smoked guinea fowl meat.

1.2. Statement of the problem

Food security issue is known to be composite in both developed and developing countries, where meat and meat products are considered as unwholesome commodities with respect to pathogens, and availability of natural toxins, adulterants and other possible pollutants (Yousuf *et al.*, 2008). Consumption of contaminated poultry meat and meat products result in high risk of foodborne diseases (Kim *et al.*, 2016). Many undesirable changes of fresh meat products marketed at refrigerated temperatures are known to occur owing to microbial growth and lipid oxidation leading to meat spoilage and overall quality reduction (Gheisari, 2011). These changes are reported to occur as result of the varied nutrient composition of meat which makes it best environment for the growth and propagation of meat spoilage micro-organisms and other food-borne pathogens (Zhou *et al.*, 2010). Monitoring and controlling of these biochemical changes during meat preservation or storage are necessary due to the reported increased demand for precooked meat products for domestic and industrial uses (Raharjo *et al.*, 1992).

Despite the high nutritional value and premium quality meat, high meat to bone ratio and limited cultural barriers on consumption of guinea fowl meat, the hygienic quality under which the meat is processed is hampered by environmental contaminants during processing and preservation. The meats are mostly processed in the open, exposing

them to hazards such as dust, vehicular fumes, flies and insects responsible as carriers of various public health diseases, and unhygienic practices from the processors and/or sellers (Adzitey *et al.*, 2015). Guinea fowl meat and its products are reported to be associated with high microbial load (Adzitey *et al.*, 2015). *Escherichia coli*, *Streptococcus* spp., *Staphylococcus* spp., *Salmonella* spp., *Proteus* spp., *Pseudomonas* spp. and *Bacillus* spp., were the bacterial species identified on the guinea fowl meats, with *Staphylococcus* spp., *Bacillus* spp., and *Escherichia coli* being the most common identified bacteria in the order of importance (Adzitey *et al.*, 2015). These pathogenic microorganisms are responsible for the several public heath diseases such as diarrhoea, cholera and dysentery.

Increasing industrial and manufacturing activities have also attributed to the occurrence of several dangerous chemical substances that are carcinogenic to human health. These chemical compounds are known to exhibit the ability to accrue in many processed foods such as smoked, fried, barbequed, or grilled foods, which result in different cancer types such as bladder, lung, gastrointestinal and skin (IDPH, 2019). Although, smoking of guinea fowl meat enhances flavour and extends the shelf life of the meat to some extent, the chemical contaminants related to smoke are deposited in the meat during processing which may lead to cancer related sicknesses similarly observed in smokers. Therefore, a pressing need for innovative techniques for decontaminating smoked guinea fowl meat and meat products requires attention.

1.3. Significance of the Study

Foodborne infections and illnesses are recognized to be main causes of disease and death globally, thereby reducing economic growth and productivity. A comprehensive

understanding and study of approaches that lead to microbial decontamination, toxins and chemical reduction and elimination are required for operational management and preservation of high quality and safe food and meat products. Among various preservation methods required for improving the microbiological safety and overall shelf life of meat and meat products, irradiation is known to be an effective technology for such purpose.

Irradiation is known to be a potent advanced oxidation processes (AOPs) employed for the decomposition of various chemical pollutants such as pesticide residues (Khalil and Al-Bachir, 2017) in food. It is also considered a potent source of energy that penetrate food and decompose carcinogenic compounds (Guieysse *et al.*, 2005; Rababah and Matsuzawa, 2002) found in processed foods such as smoked, grilled and fried products. Irradiating of food, especially gamma irradiation is well known for its long-time protection and improvement of quality and safety (Prakash *et al.*, 2014; Mahindru, 2005). Therefore, irradiating food will enhance safety and extend shelf life by inactivating pathogenic and spoilage microorganisms without deteriorating product quality.

Currently, there is little information on the impact of gamma irradiation on quality and shelf life of smoked guinea fowl meat. The present study would make available data in this regard. This study would also promote the utilization of gamma irradiation in improving quality, safety and shelf life of smoked meat, especially guinea fowl meat. Besides, the health of consumers of smoked guinea fowl meat would be improved. The local and international trade in smoked guinea fowl meat would be enhanced.

1.4. Objectives

The principal objective of the study was to use gamma irradiation as a decontaminating technique in improving the hygienic quality and shelf life of smoked guinea fowl meat. In achieving this, specific objectives of the study conducted were outlined.

1.4.1. Specific objectives

The specific objectives of this study were to:

1. Determine the effect of gamma irradiation on the nutritional quality of smoked guinea fowl meat.
2. Investigate gamma irradiation effect on the polycyclic aromatic hydrocarbons (PAHs) of smoked guinea fowl meat.
3. Evaluate the combined effect of gamma irradiation and refrigeration storage period on the shelf life of smoked guinea fowl meat.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Poultry

Globally, the consumption of poultry meat has increased over the years due to reasons such as popularity and ease of its production, nutritional benefits, fairly low price compared to other farm animals, and absence of religious constraints on its consumption (Adeyinka *et al.*, 2007). It is also regarded as a cheap way of alleviating poverty amongst resource rural poor (Saina, 2005). There is an increasing attention to nutritional value, housing systems and domestication of other avian species, although the production and consumption of chicken meat rank first (Valceschini, 2006). Guinea fowl (*Numida meleagris*), turkey (*Meleagris gallopavo*), ducks (*Anas platyrhynchos*), and pigeons (*Columba livia*) follow in the order of importance of domestic fowls in most countries. The production of guinea fowl has been on the increase among smallholder farmers' localities in most tropical areas (Anon, 1991). Thus, the increased domestication and production of guinea fowl has improved the livelihood of the poor resourced, and increased food security (Adzitey, 2013; Issaka and Yeboah, 2016).

In Ghana, the local poultry contributes significantly to household incomes and a good source of protein for many households, mostly in the rural areas (Hagan *et al.*, 2013). The local poultry (comprising of domestic chicken, guinea fowls, ducks, turkeys, and quails) have been used to promote food security, curb malnutrition among rural folks and resource poor (Issaka and Yeboah, 2016). This succeeded because these birds are

relatively hardier (tolerant to environmental stress) than exotic fowls, tolerant to disease conditions, and thus easier to keep (subsist on minimal feed supplementation (Sayila, 2009).

2.2. Guinea fowl

2.2.1. Origin and distribution

Guinea fowl (*Numida meleagris*) belongs to the family *Phasianidae* and the subfamily *Numidinae*, which is one of the six guinea fowl species found only in Africa and Arabia (Weimann *et al.*, 2016). Because of their large existence in the wild of many African countries, they are believed to have originated from West Africa, specifically the coast of Guinea hence, the name 'Guinea' (Annor *et al.*, 2012; Moreki and Seabo, 2012). Guinea fowl has been successfully domesticated in many communities and villages of Africa. It is one of the wild birds that has been domestically reared (farm-raised) together with other fowls such as chickens and ducks (MacDonald, 1992). It can either be used as an alternative poultry system, or part of the poultry system itself. The EU countries such as France, Belgium, Italy, and Scandinavian countries have shown increasing interest in guinea fowl production, of which its meat is valued for taste and nutritional properties (Beaza *et al.*, 2001), with France being the largest producer and consumer of guinea fowl (Audran, 2005). The production of the fowl has successfully proven commercially viable in the United States of America where it is raised in large quantities (Cassius and Radikara, 2013). In Ghana and other developing countries, guinea fowls have become a savored delicacy due to the

leanness of the meat, with its peculiar flavour and taste (Asare-Mensah, 2014; Mareko *et al.,* 2006).

2.2.2. Characteristics of guinea fowl

Guinea fowls are easily identified or described as having featherless heads with helmeted varieties. Their head and neck are bare, but may have wattle with male wattle being much larger than that of females (Moreki, 2009). Their distinct features include; resistant to many poultry diseases at the adult stage (Sayila, 2009), inexpensive production cost as they mostly scavenge for food, and utilise quite a large range of feed (Saina, 2005). Other characteristics include: more resistant to heat than chicken, thus require higher temperature to raise, very noisy and cannot be reared near to suburban places, it is timorous, with a more sociable behaviour than chicken, but darkness and presence of perches reduce the bird's timidity, capable of causing heavy losses, it likes to hide and remain immobile when afraid with a crowding together behaviour, and it withstands transportation better than chicken (Moreki, 2009). The grey type are known to adapt well to local climatic conditions and resistant to many poultry diseases. This helmeted guinea fowl exhibit unique characteristic features, such as shanks of slate grey colour with more or less grey-blue plumage with many rounded small white spots (Plate 2.1).

Plate 2.1. Grey and white stripped helmeted guinea fowl. *Source: Ayinpoya (2016)*

2.3. Guinea fowl industry in Ghana

The production of guinea fowls requires less capital due to their tendency to scavenge and fend for themselves (Dougnon *et al.*, 2012; Saina, 2005). As such, most farmers allow their fowls to scavenge with some supplements provided. This makes it easy to be managed by resource poor farmers. The three northern regions of Ghana (Northern, Upper East, and Upper West), traditionally rear guinea fowls on large scale although the birds have been increasingly introduced to other regions of the country. Some of these regions include; Brong Ahafo, Volta, Ashanti and Greater Accra. These birds have been known to be the commonest poultry species in the Northern Ghana (Agbolosu *et al.*, 2012). Their populace has been estimated to institute about 7.1% of the total poultry population, with the three northern regions contributing about 81% of all guinea fowls produced in Ghana (FAO, 2014).

Majority of the birds raised in Ghana are reared by subsistence farmers mostly from rural areas. As reported by FAO (2014), large-scale production of guinea fowls under

intensive management has been successful in northern Ghana, but industrial production has failed since exotic guinea fowl production has been insignificant. However, few commercial guinea farms have emerged in the southern part of Ghana (Annor *et al.*, 2012). These commercial guinea fowl farm ventures are mostly practiced by commercial farms of educational and research institutions, such as the Animal Research Institute of the Council for Scientific and Industrial Research (CSIR) of Ghana, and the Livestock Production and Research Centre (LIPRC) of the University of Ghana, Legon.

2.3.1. Breeds of Guinea fowl in Ghana

Varieties of guinea fowls are distinguished based on plumage colour: Pearl (grey), White, Black, Lavender, Blue, Lilac, Cream, Buff or Peacock (Bernacki *et al.*, 2012). Some are plain headed, plumed, crested, grey-breasted, helmeted and white-breasted. Several breeds of guinea fowl exist, but the most common are *Numida meleagris* – red-wattled guinea fowl, and *Numida ptilorhyncha* – collarette of feathers on the upper part of the neck (Binali and Kanengoni, 1998). Within the nine different helmeted guinea fowl subspecies found in Africa as reported by Moreki (2009), the "bristle-nosed guinea fowl" (*Numidia meleagris*) is common in Ghana. The grey type (helmet guinea fowl) are reared locally in most communities of Ghana, while the white, and mixed colour type are commercially raised in small numbers (FAO, 2014). In Ghana, both local and exotic breeds are reared and kept, with *Numida meleagris* (Pearl-helmeted type) being the most dominant and commonest breed (FAO, 2014).

2.3.2. Management system

Guinea fowls are easy to manage with little or no proper veterinary services, especially at adult stage, thus they are managed mostly by the resource poor farmers. The health management of guinea fowls depends largely on ethno-veterinary medicine (Moreki and Radikara, 2013).

Poultry birds in Ghana are managed under three housing systems: extensive (free range), semi-intensive (semi-free range) and intensive (deep litter or battery cage) management systems. Guinea fowls are commonly raised under the extensive and semi-intensive systems (Asare-Mensah, 2014). Due to their adaptability to diverse environmental conditions, raising small flocks of these fowls under free-range production system characterized by very low inputs and low productivity makes them attractive to farmers (Moreki and Radikara, 2013). The intensive system is however, mostly practiced by commercial entrepreneurs/farmers (Issaka and Yeboah, 2016). Housing system is usually rudimentary.

2.3.3. Marketing and distribution system

The relished delicacy and choice of guinea fowl meat for most people in the northern Ghana, has led to the increased demand in guinea fowl in other parts of the country (central and southern Ghana) (Issaka and Yeboah, 2016). Most traders in the northern Ghana, transport large numbers of guinea fowls from their localities to metropolises and big towns for sale. They sell to other traders, individual consumers, restaurants and organizations. In most cities of Ghana, it is a common sight to observe the sale of grilled guinea fowl at bars or clubs and along the roadside (Issaka and Yeboah, 2016),

while the smoked guinea fowl meats are mostly purchased from farms, markets and transit areas. The eggs of the fowls are rather sold in most rural markets during the laying season, with few offered as gifts to people or supplied to people in the cities, when demanded (Issaka and Yeboah, 2016). The fowls are usually sold at about 14-20 weeks of age under the semi-intensive system when they have attained weight range of 1.2-1.5 kg (Asare-Mensah, 2014).

2.3.4. Uses and benefits of guinea fowl

The demand for guinea fowl meat has increased rapidly across the regions of Ghana, especially in the three northern regions of Ghana, providing enormous opportunities for food and income security (Issaka and Yeboah, 2016). Most rural folks and the resource poor have been consuming this extensively raised (organic) poultry meat due to the well-known benefits compared to fowl meat raised more intensively. Some of the benefits of the organically raised guinea fowl include; toughness/firmness of the meat, strong bones, strong and peculiar aroma, and tastier (lean and flavourful) than intensively raised broilers. Also, these organic birds have high meat to bone ratio with restricted cultural barriers (Adeyinka *et al.*, 2007; Saina *et al.*, 2005). Because of its gamey flavour, guinea fowl is usually compared to game meat, and as such, preferred and better priced than chicken (Ajala *et al.*, 2007; Mareko *et al.*, 2006).

Guinea fowls also play important roles in the socio-cultural lives (contracting of marriages, welcoming mothers in-law, festivals, funeral rites, sacrifices, etc.) of the people of Northern Ghana (Teye and Adam, 2000), and contribute to household income. Generally, guinea fowls have been reported to contribute in improving food and nutritional security of the resource-poor, reducing their livelihood liability and

insecurity, and upholding gender equity (Ahuja and Sen, 2007; Otte, 2006; Dolberg, 2003).

2.3.5. Challenges faced by guinea fowl industry in Ghana

Major constraints to guinea fowl industry in the country include; high keet mortality (keet are more susceptible to diseases and/or environmental stress, unlike its adults), low productivity of local birds, inadequate access to veterinary services and drugs, unstable prices and poor management practices (Issaka and Yeboah, 2016). Also, poor storage and inadequate post-harvest technologies for proper storage remain a challenge in the meat processing industries, suggesting that not much progress has been made to improve preservation and storage mechanism (Personal comm, 2017).

2.4. Guinea fowl meat

The meat of guinea fowl is white like chicken meat (Plate 2.2A) with some dark portions around the neck, legs, wings, thighs and back (Plate 2.2B). The meat has a dark colour similar to that of game birds, clearly distinguishing it from chicken meat. Its taste is more reminiscent of pheasant, thus having a gamey flavour (Tlhong, 2008; Ajala *et al.*, 2007).

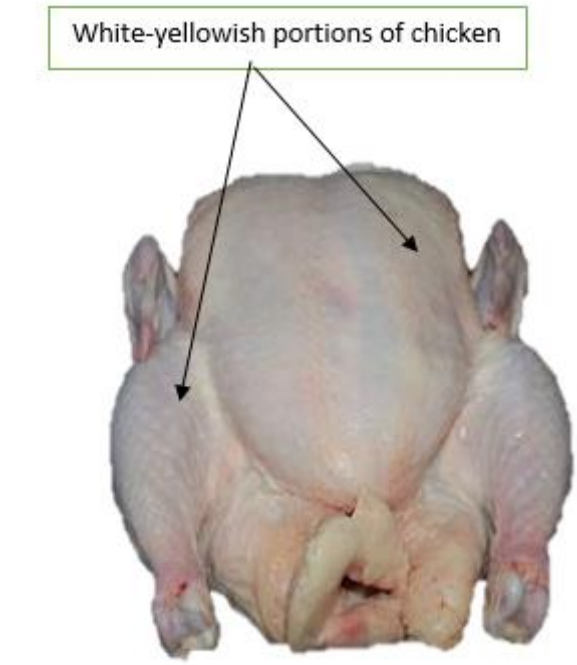

Plate 2.2. A: Chicken meat

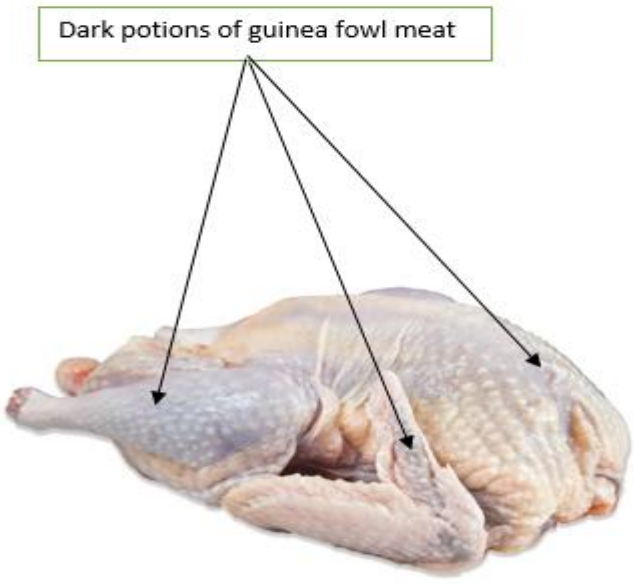

Plate 2.2 B: Guinea fowl meat

Source: Picture taken by Evelyn Otoo, 2018.

2.4.1. Nutritional quality of the meat

Nutritionally, the meat of guinea fowl is known to be rich in essential amino acids and fatty acids, low in cholesterol/calories and critical micronutrients like iron, calcium, zinc, and vitamins (thiamine, riboflavin), which are likely of preventing under-nutrition (Tlhong, 2008). Guinea fowl meat is substantially drier and leaner than chicken, having about 4% fat content as compared to 7% fat for chicken (Asare-Mensah, 2014; Nsoso *et al.*, 2003), as well as high protein content of 23% against 21% for chicken (Moreki *et al.*, 2010; Nsoso *et al.*, 2003). According to CAB International as reported by Northcutt (1997), the high protein (23%) and low fat (4%) contents, make the guinea fowl meat a good additive to the rural population's poor diet. Furthermore, its high protein content compared with major meat types such as beef (21%) and pork (21%) (Warriss, 2000), make it appealing to the health conscious people.

Guinea fowl meat contains minerals such as Calcium, Sodium, Copper, Potassium, Magnesium, Iron, Phosphorous, Zinc, Cobalt, and Manganese. The meat is said to be richer in magnesium, calcium and iron (Northcutt, 1997). Guinea fowl meats are good source of minerals with pretty high values for iron, calcium and zinc compared to chicken, ostrich and beef, especially in the darker muscle (Tlhong 2008). Chepkemoi *et al.* (2015, 2017) also reported significant amounts of calcium, iron and zinc in domestic guinea fowl meat, with calcium being the highest mineral among other indigenous and commercial fowls in Kenya.

Variation in nutritional content of the guinea fowl meat exist due to variation in factors such as feed, breed, age at slaughter, production system, sex, processing and part of the cut meat as proposed by Haunshi *et al.* (2010). Thus different studies have

been reported on the nutritional quality of the guinea fowl meat, as summarised in Table 2.1.

Table 2.1. Nutritional diversity of guinea fowl meat

Proximate composition (%)	***References***				
	Chepkemoi *et al.*(2017)*	Tlhong (2008)	Chepkemoi *et al.* (2015)*	Mareko *et al.* (2008)	Saina (2005)
Moisture	74.9	74.55	74.89	74.53 [A] 72.92 [B]	
Protein	19.5	22.60	19.45	86.65 [A] 87.24 [B]	75.4[X] 72.7[Y]
Fat	2.17	2.26	2.41		
Ash	1.00	1.01	1.00	18.15-6.6[A] 20.20-8.8[B]	9.3[X] 7.8[Y]
Carbohydrates	2.978		2.978		

**Domestic fowl, A-concrete floor housing, B-earth/soil floor housing, X-under intensive system, Y-under extensive system.*

Since protein quality is known to be measured by its capability to satisfy human requirements for amino acids (Bender, 1992), several amino acids (at least 16) are found in the meat of guinea fowl (Table 2.2).

Table 2.2. Amino acid composition (g/100 g sample) of different cuts of guinea fowl (*Numida meleagris*) meat.

Amino acids (g/100g)	**Breast**	**Drumstick**	**Thigh**
Alanine	2.11	2.10	1.86
Arginine	0.94	1.01	1.90
Aspartic acid	2.19	2.09	1.96
Cystine	0.16	0.15	0.14
Glutamic acid	2.93	2.99	2.73
Glycine	1.71	2.33	1.76
Histidine	0.59	0.44	0.41
Isoleucine	0.55	0.58	0.52
Leucine	1.59	1.56	1.44
Lysine	1.40	1.37	1.226
Methionine	0.55	0.52	0.49
Phenylalanine	0.58	0.59	0.54
Proline	0.95	1.21	1.00
Serine	1.23	1.23	1.16
Threonine	1.02	1.05	0.97
Tyrosine	0.48	0.48	0.44
Valine	0.70	0.71	0.66

Source: Tlhong (2008)

Tlhong (2008) also reported a number of fatty acids (Table 2.3) detected in different cuts of raw guinea fowl meat comprising 12 polyunsaturated fatty acids (PUFAs), 8 monounsaturated fatty acids (MUFAs) and 14 saturated fatty acids (SFAs), and total

unsaturated fatty acids (TUFAs), *n*-3 (Omega 3) and *n*-6 (Omega 6) values, P:S and *n*-6:*n*-3 ratios, and cholesterol content of different cuts for guinea fowl (Table 2.3). Whole guinea fowl meat (meat and skin) have also been reported to contain higher values of SFA and MUFA (Chepkemoi *et al.*, 2017).

Table 2.3: Total fatty acid composition (%) and cholesterol content (mg/100g) of different cuts of raw guinea fowl (*Numida meleagris*) meat.

Total	Breast	Drumstick	Thigh	Whole meat*	Breast muscle**
SFA	26.77	24.60	25.12	33.8	43.5-42.8%
MUFA	26.99	25.11	27.28	37.3	20.3-20.0%
PUFA	46.24	50.29	47.59	29.0	36.2-37.2%
TUFA	73.23	75.40	74.88		
P:S	1.74	2.07	1.92		
n-6	40.30	40.83	44.32		
n-3	4.56	6.23	2.72		
n-6:n-3	8.83	6.56	16.36		
Cholesterol	56.84	126.18	131.75		

Source: Tlhong (2008). (*) - Chepkemoi et al., (2017), (**) - Bernacki et al., (2014): fatty acids in grey and white guinea fowl breast muscle.

2.5. Food contamination

Contaminated food can be described as food that is spoiled or infected by either microorganisms or toxic substances, making it unwholesome for consumption (WHO, 2010a). Food can be contaminated from environmental sources which are mostly

anthropogenic, apart from natural ones, or microorganisms. Contamination can also come from industrial food processing and some domestic food processing such as heavy metals contamination (Wilcock *et al.*, 2004; O'Keeffe and Kennedy, 1998). Others include agrochemicals (nitrates, pesticides), veterinary drugs (antibiotics, anthelmintics, hormonal growth promoters) and packaging components (plasticisers) (O'keeffe and Kennedy, 1998).

Many food-borne diseases are due to consumption of contaminated foods. Poultry meat, considered as a major cheap source of animal protein with other essential nutrients is popularly consumed by the populace. However, such foods are mostly contaminated by both pathogenic and spoilage microorganisms, and other toxic substances (Mead, 2004). A number of food-borne outbreaks have been associated with consumption of poultry meat (WHO, 2010b; Wanger, 2008; Ho *et al.*, 1995). Other hazardous agents receiving attention from policy makers include mycotoxins and antibiotic drug residues in poultry meat (O'Keeffe and Kennedy, 1998).

2.5.1. Microbiological contamination

Contamination of foods by microorganisms are the commonest, mostly found in the environment (Hammond, 2010), and a major public health concern worldwide (Cohen *et al.*, 2007). Bacteria, viruses, fungi, prions, and parasites are some of the organisms responsible for food-borne illnesses (Mead, 2004; Mead *et al.*, 1999). Contaminated food causes several acute and life-long diseases, ranging from diarrhoea (one of the leading infectious diseases) to various forms of cancer (WHO, 2010b). Greater risks of contracting these illnesses are from mishandling of food,

eating raw or undercooked food, and food with poor package and storage mechanisms (Akbar and Anal, 2011).

2.5.1.1. Bacterial contamination

According to Wardlaw (2003), the greatest health risk currently from food is contamination from bacteria with a lesser effect from fungi and parasites. Harmful bacteria are the most common causes of food-borne illnesses, and almost all reported cases are caused by toxins produced by the bacteria (Akbar and Anal, 2011). These toxins are formed in the food before it is eaten, which cannot be detected by taste, aroma or colour (Akbar and Anal, 2011). Diarrhoea, typhoid, cholera and hepatitis are some of the bacterial food-borne illnesses (Akbar and Anal, 2011; Mead *et al.*, 1999). Symptoms of these diseases may include vomiting, fever, dehydration, stomach upsets, nausea and abdominal cramps. Gram-positive spore-forming bacilli, bacteria from enterobacteriaceae family, and other micrococci often get attracted to meat held at room temperature (Roca and Incze, 1990). Though refrigeration suppresses these microbes, it allows the growth of other organisms such as *Listeria* and *Pseudomonas* (Marshall *et al.*, 1991).

Bacteria multiply extremely fast (several million in 8 h and thousands of millions in 12 h) under suitable temperature or unsafe temperature for food (Lund *et al.* 2000). Most food poisoning are caused by food-borne pathogens such as *Staphylococcus aureus*, *Salmonella* spp., *Bacillus cereus*, entero-pathogenic *Escherichia coli*, *Streptococcus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Shigella* species, *Vibrio* species such as *Vibro parahaemolyticus*, *Yersinia enterocolitica* and *Aeromonas hydrophila* (Murrell *et al.*, 1993; Chung and Murdock,

1991). These organisms can also be used to determine the hygienic quality of poultry meat. The pathogens are commonly found on raw meat though they can be present when meat is processed and mishandled. These pathogens can be prevented by controlling and reducing initial number of bacteria present and growing respectively, using appropriate treatment mechanisms and avoiding recontamination (Wanger, 2008). Wanger (2008), outlined some pathogenic microorganisms that are of food safety concern:

1. ***Bacillus cereus***: This is a spore-forming organism resistant to many physical and chemical treatments. They are found mostly in starchy foods and dry foods (herbs and spices). Ingestion of intoxicated foods by this organism results in symptoms of mild diarrhoea and nausea within 12-24 h. The bacteria can be damaged by normal cooking but its spores are heat-stable and resistant to other physical and chemical treatments.
2. ***Clostridium perfringens***: These are also spore-producing pathogens of the species of *Clostridium*. They form spores when conditions are unfavourable. They are mostly found on meat and poultry dishes and prefer low oxygen atmosphere. Symptoms of their intoxication are cramps and diarrhoea with vomiting or fever within 12-24 h. The bacteria can be suppressed and damaged by normal cooking, but becomes heat-stable in its spore state.
3. ***Staphylococcus aureus***: They are facultative, anaerobic gram-positive cocci. They occur singly, in pairs or irregular clusters. *S. aureus* is a species of *Staphylococcus* commonly found on body surfaces such as the skin and superficial wounds. They are also found in the nasal cavity and throat of 30-50% of healthy people. Mishandling of food item results in contamination of this microorganism. Diarrhoea with no fewer within 4-6 h of ingestion,

vomiting and nausea are some of the symptoms of intoxication. Heat can destroy the bacteria but its toxin is heat-stable.

4. ***Salmonella* spp.**: These are characterized as Gram-negative, rod-shaped bacterial of the family of *Enterobacteriaceae*. Francis *et al.* (1999) reported these organisms to be facultatively anaerobic, capable of survival in low oxygen atmospheres. Species of such pathogen include: *S. typhimurium, S. enteritidis, S. saint-paul, S. Heidelberg,* and *S. Montevideo* (Francis *et al.,* 1999). They are abundant in faecal materials, sewage and sewage-polluted water, and consequently in soil. Naturally, they are associated with the bodies of all animals. Foods of the animal origin are the primary vector of *Salmonella* towards human. Symptoms of ingestion of these organisms includes diarrhoea, abdominal pain, vomiting, nausea, and mild fever.
5. ***Escherichia coli**:* This is a type of species of the Enterobacteriaceae genus. It is a common inhabitant of the gastrointestinal tract of mammals. The pathogenic strain (enterohaemorrhagic *E. coli* 0157:H7) have emerged as highly significant foodborne pathogens. The primary source of *E. coli* has been reported to be related to the bovine gastrointestinal tract (Khanna *et al.,* 2008; Doyle, 1990). Hence, making contamination of food significantly important. Symptoms such as gastroenteritis, haemorrhagic colitis, and haemolytic uramic syndrome have been documented (Martin *et al.,* 1986).
6. ***Campylobacter jejuni**:* *Campylobacter* are zoonotic pathogens, primarily associated with the intestinal tracts of wild and domestic animals (Thomas *et al.*, 1995), which are dispersed throughout the environment by birds, flies and surface water. These are gram-negative, spiral microaerophilic bacteria, emerged as a major human gastrointestinal pathogen (Ketley, 1997).

Members of these *C. jejuni* survive at refrigeration temperatures for extended periods within limited nutrient environments. This characteristic specifies their potential importance with respect to refrigerated food. Poultry are known to be the main carriers of *C. jejuni* (Kozaĉinski *et al*., 2006), and contamination can occur during slaughter if birds are not initially affected. Gastrointestinal symptoms and Guillain Barre Syndrome are common disease symptoms associated with the organism, with the later considered by symmetrical ascending paralysis (Ho *et al.*, 1995).

2.5.2. Toxic/carcinogens contamination

Toxins are not produced by microorganisms only, but also from the environment through human activities (e.g. mining, construction, waste generation and disposal, etc.). The toxins from hazardous agents (e.g. fumes, dust, smoke, organochlorine and pesticide residues, etc.) released into the environment pollute foods for consumption (O'keeffe and Kennedy, 1998). Industrial and domestic processing of foods expose them to a number of these hazardous agents which when consumed becomes a threat to human health. Exposing food to intense heat source during processing releases certain compounds that can lead to cancer (long-life effect) when consumed on daily basis or serve as main meal (Jimenez-Colmenero *et al.*, 2001). One of such compounds found in most processed foods is polycyclic aromatic hydrocarbons.

2.5.2.1. Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs), also known as polynuclear aromatic hydrocarbons are group of compounds formed from incomplete combustion of organic

matter or carbonaceous materials obtained from the environment and natural resources such as air, soil, water, and food (Suchanová *et al.*, 2008; SCF, 2002; WHO, 1998). They are also formed by pyrolysis of organic matter during various industrial processes (EFSA, 2008). These large groups of organic compounds contain two or more fused aromatic rings of hydrogen and carbon atoms, without heteroatoms (Doris and Ken, 2009; Anyakora and Coker, 2007). They are known to be carcinogenic, micro pollutants, toxic and ubiquitous in the environment (Khalil *et al.*, 2016; Simko 2002), thus play key role in imposing health risk on humans. In 2001, PAHs ranked 9th on the list of most threatening compounds to human health (King *et al.*, 2002). Apart from food being the main source of PAHs exposure to humans, exposure via inhalation of polluted ambient and indoor air, ingestion of house dust, and dermal absorption from contaminated soil and water are minor routes (WHO, 1998).

The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has evaluated the carcinogenicity of some PAHs based on evidence in humans and experimental animals (IARC, 2013). The IARC's classification of some PAHs is summarized in Table 2.4.

Most of the PAHs evaluated are classified as:

(a) Group 1: the agent is carcinogenic to humans

(b) Group 2A: the agent is probably carcinogenic to humans

(c) Group 2B: the agent is possibly carcinogenic to humans

(d) Group 3: the agent is not classifiable as to its carcinogenicity to humans

Table 2.4: Genotoxicity and Carcinogenicity of some PAHs

Common name	Genotoxicity[1]	EU priority PAHs	IARC group
Acenaphthene	Questionable	-	Not yet evaluated
Acenaphthylene	Questionable	-	Not yet evaluated
Anthracene	Negative	-	3
Benzo[a]anthracene	Positive	*	2A
Benzo[b]fluoranthene	Positive	*	2B
Benzo[k]fluoranthene	Positive	*	2B
Benzo[g,h,i]perylene	Positive	*	3
Benzo[a]pyrene	Positive	*	2A
Chrysene	Positive	*	3
Dibenz[a,h]anthracene	Positive	*	2A
Fluoranthene	Negative	-	3
Fluorine	Negative	-	3
Indeno[1,2,3,-cd]pyrene	Positive	*	2B
Naphthalene	Positive	-	2B
Phenanthrene	Questionable	-	3
Pyrene	Questionable	-	3

*Source: International Agency for Research on Cancer (IARC) 2013. * -EU Priority PAHs*

None of the PAHs was classified carcinogenic (group 1), until recently, IARC categorized processed meat and red meat as carcinogenic and probably carcinogenic to humans (groups 1 and 2A) respectively, based on epidemiological studies reporting correlations with cancer (Bouvard *et al.*, 2015).

2.5.2.2. PAHs occurrence in food

The highest intake of PAHs has been reported to be associated with their occurrence in food (Suchanová *et al.,* 2008), thus being the main source of exposure to humans and non-smokers (SCF Annex, 2002). The food types include; fruits and vegetables, cereals and grains, and oils (Moret *et al.,* 2005; Simko, 2002; Guillen *et al.,* 1997), duck meat (Chen and Lin, 1997), smoked cheese (Pagliuca *et al.,* 2003), fish (Palm *et al.,* 2011; Serden *et al.,* 2010; Wretling *et al.,* 2010; Moret *et al.,* 1999), pork and beef (Chung *et al.,* 2011) and other protein food – meat, cowskin, fish and crayfish (Taiwo *et al.,* 2019). It has been well documented that PAH concentrations are high in most fishes and meats that have undergone some thermal treatments at high temperatures. That is, processed meat products were found to contain high amount of PAHs (Chen and Lin, 1997). Also, foods that have been processed in direct contact with combustion gases such as grilling, roasting, barbecuing, smoking, baking or frying as well as drying can results in high levels of PAHs and as major source of PAHs contamination in food (CCFAC, 2005; SCF, 2002).

Contamination of food with environmental PAHs has been reported to depend on some physical and chemical properties of PAHs, namely solubility in water and fats/oils, volatility, chemical reactivity, and biotic and abiotic degradability (Guieysse *et al.,* 2005; Abo-El-Seoud *et al.,* 2004). The amount of these compounds in smoked meats and fishes have also been reported to depend on factors such as temperature, oxygen accessibility, fat content, duration of treatment/cooking, distance from the source of heating, type of combustible material used (Visciano *et al.,* 2006; WHO, 1998), and whether melted fat is allowed to drip onto the heat source (SCF, 2002; Nawrot *et al.,* 1999). It has been reported that people with a diet rich in roasted,

barbecued and smoked food may have substantial intake of PAHs (SCF Annex, 2002), although these foods contribute a smaller part of PAHs intake. This brought in mind the detection and development of extenuation approaches to reduce their contents in food for food safety.

2.6. Preservation of meat

Keeping food under safe conditions to extend the shelf life of the food product whilst maintaining its nutritional quality and controlling microbial growth can be described as food preservation. Preservation of food has been with man since antiquity (Pamplona-Roger, 2006). Storage methods, packaging systems, and processing of the food prior to storage (Wardlaw, 2003) among others, been some diverse ways of preserving food. Some of the food preservation methods practiced for ages include; drying, salting, smoking, fermentation, pickling, bottling and canning (Thurmond, 2006). Modern and innovative methods of preservation include; pasteurizing, chilling, freezing, addition of chemicals/antioxidants and irradiation (Desrosier and Singh, 2011).

In Ghana, drying, salting and smoking have been traditionally practiced over the years among many rural folks. These methods are basically used for preserving most meat and fish products (e.g. salted tilapia fish (Koobi) and dried meat (Jerky)). Chilling and freezing are commonly used for fresh and freshly processed meat and fish products. Storing food at refrigeration temperatures (3–5 ^{o}C) slows the growth of microorganisms and biochemical changes occurring in storage food, unlike the ambient temperatures where microbial and enzymatic reactions proceed rapidly (Lund *et al.*, 2000).

2.6.1. Smoking

Smoking simply means slowly cooking food indirectly over a fire. This can be done using a smoker, covered grill or grilling pan, open wood smoke, smoke house, and commercial liquid smoked flavourings (Martinez *et al.*, 2007). The product quality of smoked food (mostly meat and fish) is reported to be affected by wood fuel used (Benjakul and Aroonrueng, 1999). Smoking meat products has been one of the food technologies used for food preservation since antiquity (Djinovic *et al.*, 2008). This technology uses the special effects of diverse sensory active compounds contained in smoke to aromatize food products. These compounds are various aromatic hydrocarbons and their alkylated derivatives (Abdallah, 2013). Smoked meat products still remain a substantial part of the human diet because of their exceptional taste, high nutritional value, and large variety of available products (Kim *et al.*, 2014). Stolyhwo and Sikorski (2005) stated that smoke composition and processing conditions affect sensory quality, shelf life, and wholesomeness of the product.

In the traditional setting, occasional re-smoking of smoke-dried fishes and meat product are mostly carried out to maintain dryness and control mould attack and insect infestation during storage, for enhanced shelf life (Ghana Postharvest Fisheries Overview, 2003). However, this occasional re-smoking condition do not keep the meat safe as a result of frequent handling, insect infestation and microbial decomposition as had been seen in smoked-dried fishes in the country (Ghana Postharvest Fisheries Overview, 2003; Fialor *et al.*, 2002). As such, the quality and safety of the food (e.g. meat) are reduced during storage. Hence, the use of post-processing method such as modifications of existing methods and the use of innovative techniques will be beneficial to the food industry.

Smoke treatment of food products (Song *et al.*, 2009; Djinovic *et al.*, 2008; Stolyhwo and Sikorski, 2005) and the effect of different smoke agents on the quality of food products (Oduor-Odote *et al.*, 2010) have been reported. The quality of smoked meat and fish products is influenced by raw meat and fish materials (Cardinal *et al.*, 2001), salting and/or brining concentration (Alcicek and Atar, 2010), processing conditions (Duffes, 1999), smoke composition (Stolyhwo and Sikorski, 2005), smoking method (Cardinal *et al.*, 2006), and smoke agents (Siskos *et al.*, 2007).

2.6.1.1. Smoking methods

Smoking can be categorized as traditional or modern, depending upon the smoke deposition into the food products. In the traditional technique, oven is used to generate smoke, which is formed directly by burning wood chips or sawdust wood (Visciano *et al.*, 2008; Stolyhwo and Sikorski, 2005). In the modern technique, an electric field acts on ionized smoke particles which quicken the smoke deposition or the use of commercial liquid smoke flavourings (Martinez *et al.*, 2007; Duffes, 1999).

Moreover, smoking methods are divided according to smoking temperature namely hot smoking, warm smoking or cold smoking (Stolyhwo and Sikorski, 2005; Rørvik, 2000; Duffes, 1999). The cold smoking process uses temperatures ranging from 15-30℃ (75-85% relative humidity) (Arason *et al.*, 2014), and final salt content of at least 3.5% water phase salt (WPS) (University of Florida, 2004). Warm smoking (traditional smoking) process uses 30-50℃ (50-70% relative humidity), and a high temperature smoking process (hot smoking method) uses 50-80℃ (40-50 % relative

humidity). The internal product temperature of at least 62.8 ^{o}C for at least 30 min must be obtained (University of Florida, 2004).

2.6.2. Food irradiation

Exposing food and food products to ironizing energy from radioactive sources in order to control and/or eliminate spoilage and pathogenic organisms from food is described as food irradiation (Jouki and Yazdi, 2014). The energy can be in a form of rays or speed particles. Appiah (1999), also explained food irradiation as exposing food (either packaged or un-packaged) to ionizing radiation from sources like gamma rays (Cobalt-60), X-rays or electrons. Wardlaw (2003) defined this radiation technology as passing of gamma rays through food to destroy cell membranes, break down DNA, link proteins and change some cell functions that can lead to food spoilage. Food irradiation is a physical way of preservation (cold processing) comparable to processes such as heat pasteurization, cooking, canning and freezing. The process does not make food radioactive as it is reported not to induce radioactivity in food by either gamma irradiation or electron beams up to 10 MeV (Farkas, 2004). This makes it a promising innovative food safety technology for improving hygiene and storage life of commodities.

2.6.2.1. Radiation sources

Ionizing radiation occurs when one or more electrons are dislodged from the electronic orbital of atoms and/or molecules and converting them to electrically-charged ions (Mohd Dahlan, 2001). These radiations can be produced by three

different techniques namely gamma ray processing, high energy electron called e-beam, and X-ray processing. Radioisotopes/radionuclides such as Cobalt-60 and Cesium-137 emit gamma rays, while e-beam and X-rays are generated by electron accelerator and X-ray machines respectively (IAEA, 2002; Mohd Dahlan, 2001). X-rays (maximum energy of 5 MeV), electron accelerators (maximum of 10 MeV) and gamma rays produced from radioisotopes cobalt-60 (1.17 and 1.33 MeV) and cesium-137 (0.662 MeV) are irradiation sources approved internationally for food processing (CAC, 1984). These energies are too low to induce radioactivity in food or any material (Farkas, 2004). Cobalt-60 (with a half-life of 5.27 years) gamma rays are entirely used for irradiation of foods and in treating full boxes of fresh or frozen foods (ICGFI, 1999).

2.6.2.2. Mechanism of Radiation damage

Irradiation works on the principle of energy discharge from electrons (Mohd Dahlan, 2001). Charged electrons and free radicals are reactive species produced in the product being treated, which interact with chemicals in cells and interrupt their division. These species also react with other food components. The radiolytic products of irradiation and other molecules, usually made of free radicals formed from water causes direct and indirect actions on cell (Lobo *et al.*, 2010; Farkas, 1998), as seen in Figure 2.3. The indirect action (Figure 2.3) of gamma rays which interact with other molecules or atoms (usually water), producing reactive molecules, such as hydroxyl radicals, hydrogen peroxide and hydrogen atoms cause damage to DNA (Diehl, 1995) and other similar effects to those resulting directly by radiation (Ahn and Lee, 2006; Dickson, 2001; WHO, 1999). Thus microorganisms can be inactivated by the

impairment of its DNA and other organelles of the cell (Diehl, 1995). This damage leads to cell death once the double-strand DNA breaks, and cannot be repaired by the cell (Hall and Giaccia, 2006).

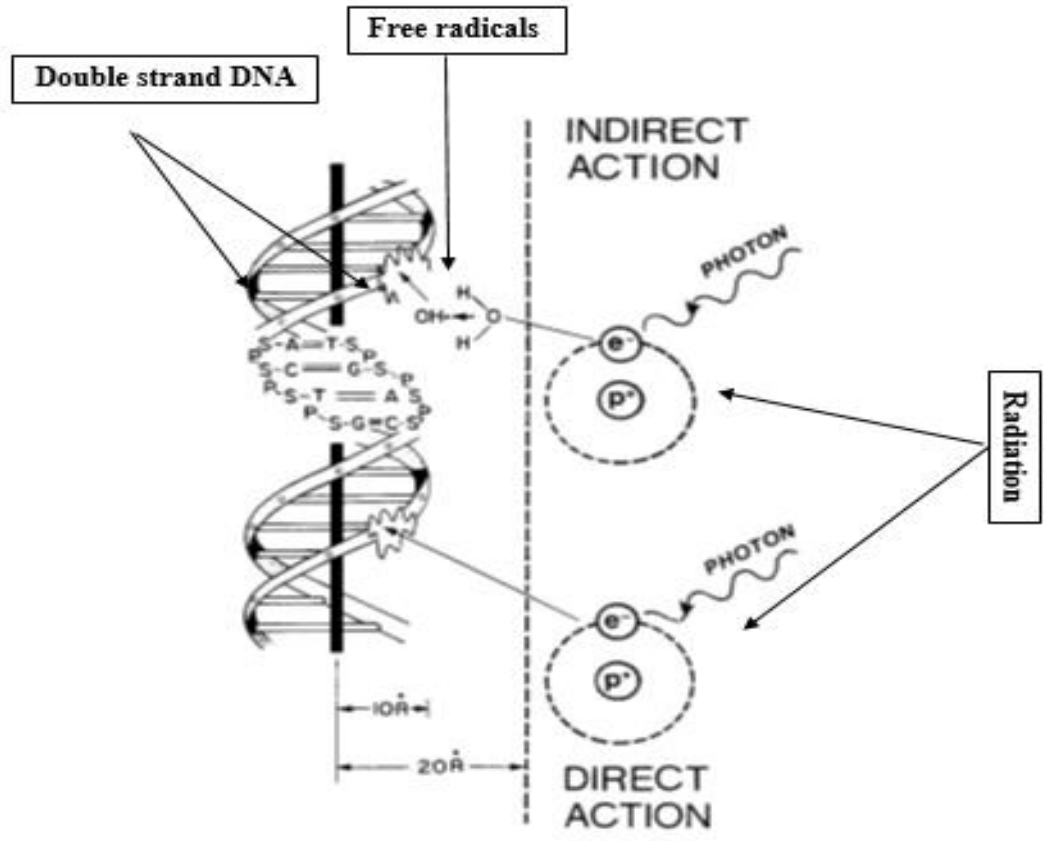

Figure 2.3: Mechanism of radiation damage

Source: RSSC Biological effects of ionizing radiation (2011)

The direct hit (direct action) by radiation on the DNA is fairly unusual, due to the small size of the DNA helix (a diameter of about 2 nm) (RSSC, 2011). However, damage from indirect action is more common (especially for radiation that has a low specific ionization). This is because, free radicals produced are able to diffuse some

distance in the cell, attacking critical targets such as the DNA (RSSC, 2011), hence, are more lethal than the direct action.

2.6.2.3. Purpose of food irradiation

Irradiation has been considered as one of the most efficient non-thermal technological process and preservative methods for the control and elimination of microorganisms in food (Mostafavi *et al.,* 2012). Insect disinfestation, sprout inhibition of root and bulb crops, delay of ripening or senescence, and the extension of shelf life of perishable agricultural produce and food products, are among the purpose of food irradiation. Generally, irradiation is used to improve the safety of food products, hence enhancing or maintaining the quality of food without obvious effect on its physical state (Mostafavi *et al.*, 2012). Marcotte (2001) reported that, the irradiation technique can be used as quarantine treatment for a variety of fruits, vegetables, cut flowers, and animal origin products to facilitate international trade. Rahman (2007) summarized the main advantages of food irradiation as follows:

- It is highly effective, efficient and environmentally friendly.
- Fresh or frozen products can be treated in their final packaging.
- Little or no heating of the food and therefore negligible change to sensory characteristics.
- Changes in nutritional value of foods are similar with other methods of food preservation.
- Processing is automatically controlled and has low operating costs.
- Gamma radiation is highly penetrating reaching all deep places.

- Irradiation has considerable potential to increase international trade in agricultural commodities.

2.6.2.4. Radiation dose and Dosimetry system

An essential quantity in radiation processing is the amount of energy that is absorbed by the medium termed absorbed dose. Radiation absorbed dose (D) may be defined as the energy deposited (ΔE_D) by ionizing radiation to a mass (Δm) of matter in a given volume elements. Simply, it is the amount of energy absorbed per unit mass of the irradiated products (Mohd Dahlan, 2001). Its standard unit is the "Gray" (Gy) or kilo gray (1 kGy = 1000 Gy). That is, 1 Gy equals 1 Joule of energy absorbed in a mass of one kilogram (EFSA, 2011). The old unit of measurement was "rad" which equals 100 ergs of energy absorbed per gram of matter (100 rads = 1 Gy) (Diehl, 1995; Olson, 1995).

Dosimetry, can be defined as the mean energy (dE) imparted by ironizing radiation to the matter in a volume element divided by the mass (dm) of that volume element (IAEA, 2002). Radiation dosimetry is thus the measurement of the absorbed dose in matter and products resulting from the exposure to radiation (Codex Alimentarius Commission [CAC], 2003). In process validation and process control in food irradiation, a documentary evidence that seeks that irradiation process has achieved the desired results is a well characterized reliable dosimetry system that is traceable to national and international dosimetry standards (IAEA, 2002). Dosimetry provides important function where large absorbed doses and dose rates are to be measured (Mostafavi *et al.*, 2012). That is, when dosimeters are exposed to radiation, they undergo physical or chemical change in properties that can be recorded. Hence, after

irradiation, the dosimeters are removed and read using a UV spectrophotometer or potentiometer (Mohd Dahlan, 2001).

Dosimetry systems can be classified on the basis of their intrinsic accuracy and applications. Dosimeters are mainly grouped into four namely; primary standard, reference standard, transfer standard and routine/working dosimeters (ASTM, 2000).

Primary standard dosimeters (e.g. ionization chambers and calorimeters) enables an absolute measurement of absorbed dose to be made with reference only to SI base units and do not require calibration (IAEA, 2002).

Reference dosimeters (e.g. Fricke, ceric-cerous, dichromate, ethanolchrobenzene (ECB) and alanine dosimeters) require calibration against a primary standard (IAEA, 2002).

Routine standard dosimeters are commonly used for dose-mapping and process monitoring for quality control in radiation processing facilities. Examples include poly methyl methacrylate (PMMA), ECB, ceric-cerous, radiochromic and cellulose triacetate (CTA) films (IAEA, 2002).

Transfer standard dosimeters are generally reference standard dosimeters that have characteristics meeting the prerequisite of a particular application, and are used for transferring dose information from an accredited or national standards laboratory (IAEA, 2002). Verified dosimetry systems have been extensively used to perform radiation measurements, quality control and validation of processes (Moreno *et al.*, 2008).

2.6.2.4.1. Radiation dose application in food and meat products

Radiation doses have been classified as low, medium and high dose applications (IAEA, 2002). A dose range from 0.1-1 kGy is said to be a low radiation dose which is mostly used for sprout inhibition of bulbs and tubers, delay fruit ripening, and insect disinfestations (EFSA, 2011).

Medium dose ranges from 1-10 kGy have been used to enhance the quality of food through substantial reductions in microbial numbers (Padua, 2009; IAEA, 2002). Padua (2009) and IAEA (2002) documented the above dose range (1-10 kGy) to be used to extend the shelf life of fresh meat, poultry and seafood. Destruction of non-spore forming pathogenic bacteria in fresh or frozen foods, and reduction in viable counts of microorganisms in spices and other dry ingredients to reduce contamination of food are irradiated with the medium dose range (EFSA, 2011).

High dose application ranges from 10-30 kGy or as high as 70 kGy (WHO, 1999). These dose ranges are effective for microbial decontamination of dried food products (spices, herbs, dried vegetables and fruits and seasonings). Also, the high dose ranges are used for sterilization of food products meant for immune-compromised patients and food meant for extension of shelf life of precooked or enzyme activated food products in hermetically sealed containers (IAEA, 2002).

2.6.2.4.2. Radiation dose effect on nutritional components of food

Although ionizing radiation produces chemical changes by primary and secondary radiolysis effects, nutritional constituents of food are not significantly affected by low-medium (1-10 kGy) dose ranges (Diehl, 1995; Diehl *et al.*, 1991). The effect of

chemicals produced by ionizing radiation depends on factors such as absorbed dose, dose rate, temperature, presence or absence of oxygen, and radiation facility type (Mostafavi *et al.,* 2012). Also, the physical status of food (solid, liquid, and powder), the composition, and the state of the food (frozen, fresh or cooked) influence the reactions induced by radiation (IAEA, 2009). Radiation doses above 10 kGy have been found to cause structural degradation of fibrous carbohydrates and rancidity of lipids (Brewer, 2009; Miller, 2006). Irradiation, is however, not known to alter the elemental (mineral) composition of food (Hajare *et al.,* 2014; FDA, 1997; Diehl, 1995; Diehl *et al.,* 1991), but, the impact of ionizing radiation on major food components (proteins, carbohydrate, and lipids) can be detrimental (Al-Bachir and Zeinou, 2014; Al-Bachir, 2013).

2.6.2.4.3. Radiation dose effect on microbiological quality of meat

Pathogens such as *Salmonella* and *Campylobacter* commonly associated with food poisoning are inactivated by some low irradiation doses. Low ionizing radiation doses (< 3.0 kGy) have been reported to eradicate or significantly decrease the population of common enteric pathogens such as *E. coli*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter jejuni*, *Listeria monocytogenes* and *Aeromonas hydrophila* associated with meat and poultry products (Thayer, 1995). Also, many studies have indicated that irradiation at doses of 3 kGy should yield 2 to 5 log10 reduction of pathogenic, non-spore forming bacteria (Lim *et al.,* 2007; Guinebretiere *et al.,* 2003). Balamatsia *et al.* (2006) reported a pronounced reduction of bacteria population (e.g. viable bacteria and lactic acid bacteria) at a dose of 2 kGy in poultry meat. Also, yeast and mould, *enterobacteriaceae* and pseudomonads have been

completely eliminated in poultry meat (Adu-Gyamfi *et al.*, 2008; Quattara *et al.*, 2001). Maximum permitted irradiation doses of 4.5 and 7.0 kGy for red meat and poultry, respectively have been documented (ISIRI, 2008). Studies have also shown that irradiation can reduce the multiplication of coliforms, *Esherichia coli*, *Psychrotrophs*, *Salmonella* and *Campylobacter* on poultry meats (Lewis *et al.*, 2002). In a variety of ready-to-eat food products, Sommers and Boyd (2006) have demonstrated that doses of 2 to 4 kGy inactivate food-borne pathogens including *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7 and *Yersinia enterocolitica*.

2.6.2.4.4. Radiation effects on sensory properties of meat

There are ample literatures on the effects of ionizing radiation on the sensory characteristics of poultry meat (Gomes *et al.*, 2003; Lewis *et al.*, 2002; DeFeliz *et al.*, 2002; Millar *et al.*, 1995). A 2 kGy dose of irradiation has been reported to reduce appreciable number of microorganisms but, prolonged storage period after irradiation reduce the sensory quality (texture, flavour and overall acceptability) of poultry meat significantly (Lewis *et al.*, 2002). Formation of free radicals during irradiation has effect on the sensory quality of poultry meat, as more of these free radicals react with molecules of the food resulting in compounds with undesirable odour and taste (Mostafavi *et al.*, 2012). These free radicals are more profound in fresh meat and freshly pre-cooked meat thus, undesirable organoleptic properties by irradiation is currently known to attribute to high-fat products (Norhana *et al.*, 2010). Lacroix *et al.* (2000) have reported that meat redness and texture of irradiated loins especially packed under vacuum are relatively well preserved during longer storage period.

Colour is probably the most important feature that most customers use when making buying decisions of product as they base their selection on visual appearance. This is because it is the first attribute seen by the consumer. Consumers associate freshness to meat and meat product which decide willingness to purchase or not (Northcutt, 1997). Poultry meat and other meat product colour have shown variety of responses with irradiation (DeFeliz *et al.*, 2002; Luchsinger *et al.*, 1996; Millar *et al.*, 1995). As reported by Nanke (1998), research has shown that irradiation can shift the colour of meat from acceptable to unacceptable at low doses (Lambert *et al.*, 1992). These authors demonstrated that myoglobin molecule was denatured by irradiation, and that irradiation-induced colour changes of myoglobin are as a result of oxidation/reduction reactions catalyzed by radiolytic products.

Tenderness as a measure of texture has been considered the most important palatability trait of meat quality. For shelf life extension purposes, meats have been tenderized to some extent by sterilizing doses (Muchenje *et al.*, 2009; Hashim *et al.*, 1995).

Another meat quality that consumers used to determine acceptability of meat and meat products is flavour. It is determined by both taste and odour/aroma, but during consumption of food, it becomes difficult to distinguish between them (Northcutt, 1997). This attribute produces undesirable quality changes in food that has been irradiated (Nam *et al.*, 2003). Such undesirable changes include; lipid oxidation, off-flavour and changes in colour that affect consumer acceptability of product (Nam *et al.*, 2003). Nanke (1998) reported that evidence showing formation of irradiation flavor is dose dependent and the threshold dose for a detectable flavor varies with species.

2.6.2.4.5. Radiation effects on physicochemical properties of meat

Lipid oxidation as a measure of rancidity in meat is known to be affected by irradiation. Lipid oxidation has been reported to increase as storage time and level of irradiation increase (Marapana and Wijetunga, 2009). The overall physiochemical properties of pork loins appeared to be relatively less affected by 6 kGy dose (Lacroix *et al.*, 2000). An *et al.* (2017) reported minimum effects of e-beam irradiation (1.5, 3 and 4.5 kGy) on the physiochemical properties of smoked duck meat at 4 ^{o}C storage. These authors reported significant differences of pH, peroxide value (POV) and thiobarbituric acid reactive substances (TBARS) with respect to the different doses and storage in the smoked duck meat.

2.6.2.4.6. Radiation effects on chemical contaminants in meat

Though animal-origin food products are known to pose serious threat to public food safety through microbial loads and their contaminants, chemical contaminants from the environment have found their way into the food system (An *et al.*, 2017). All foods are liable to some form of contamination from a number of resources, and poultry meat and meat products are no exemptions.

Chemicals and natural compounds with hazardous properties in detectable or low concentrations have been identified in meat. Chemical residues such as veterinary drugs, environmental pollutants (such as dioxins, pesticides, and phthalates), natural contaminants (mycotoxins,) and phytosanitary substances are among the most hazardous compounds that accidentally contaminate poultry products during production, processing or marketing phases (Filazi *et al.*, 2017; Sireli *et al.*, 2015; Di Stefano and Avellone, 2014). Other chemical contaminants such as toxic elements

(e.g. Arsenic, Lead and Cadmium), persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), phthalates and radioactive substances (^{131}I, ^{137}Cs and ^{134}Cs) have been found in poultry, fish, meat and meat products (Filazi *et al.,* 2017; An *et al.,* 2017; Manabe *et al.,* 2016; Brandhoff *et al.,* 2016).

Among other non-thermal technologies for postharvest decontamination of meat and poultry, irradiation has been a promising tool for decontamination of microbial and other contaminants. The effectiveness of ionizing irradiation on food microbial inactivation is well documented (Molins *et al.,* 2001; Murano, 1995), however, its effectiveness on chemical contaminant have been inadequate. Gamma irradiation is considered one of the efficient emerged technologies for the removal and elimination of chemicals such as PAHs in the environment (Abo-El-Seoud *et al.,* 2004; Kim *et al.,* 2000). It is also a potent advanced oxidation processes (AOPs) employed for the decomposition of various pollutants such as pesticide residues (Khalil and Al-Bachir, 2017). Recently, gamma irradiation has been used to decompose PAHs in foods such as cereals, grains and oils (Khalil and Al-Bachir, 2017; Khalil *et al.,* 2016; Khalil and Al-Bachir, 2015), but limited studies have been documented for poultry and meat products.

2.7. Hurdle technology in meat preservation

Combining different processing and/or preservation methods in preserving meat and meat products has been successfully achieved in prolonging shelf life, maintaining and/or enhancing the safety and quality of meat (Marapana and Wijetunga, 2009; Cambell-Platt and Grandison, 1990). This combination treatment is described in meat preservation as hurdle technology (HT). The use of this combination treatment allows

reduction in the extreme use of any single technique (Gould, 1996). Leistner (2000) reported that hurdle technology can ensure stability, microbial safety, and sensory quality of food. Thus, this HT has been reported to be developed to achieve particular objectives in terms of both microbial and organoleptic quality (Lawrie and Ledward, 2006). Temperature, acidity, redox potential, water activity, preservatives, and irradiation among others have been widely reviewed, with the stated examples being the most important hurdles used in food preservation (Leistner, 1999).

2.7.1. Gamma irradiation and combination treatment in poultry meat

Irradiation, when used alone, may cause undesirable sensory and chemical changes in some foods depending on the absorbed dose and the conditions of irradiation (Thakur and Singh, 1995). In other to avoid these detrimental effects of irradiation, it is recommended to combine irradiation with other preservation methods such as heating, cryogenic temperature and modified atmosphere or vacuum packaging (Marapana and Wijetunga, 2009). Irradiation with a number of combination methods has successfully increased the antimicrobial efficacy whilst minimizing unwanted organoleptic effects (Kim *et al.*, 2014; Cambell-Platt and Grandison, 1990). For example, sensory changes on high-fat products are known to be reduced by vacuum packaging associated to refrigeration (Zhu *et al.*, 2009). Also, irradiation renders surviving microorganisms sensitive to other sources of external stress thus, its combination with other conventional food preservation techniques provide a synergistic antimicrobial effect (Quattara *et al.*, 2001).

Irradiation doses above certain thresholds have been reported to induce undesirable changes in sensory quality of food (Lewis *et al.*, 2002; ICGFI, 1999), and thus low

doses are required if other preservative methods are combined for treatment. As such, low irradiation doses are effective in ensuring the microbiological stability of products with minor probabilities of changes in nutritional and/or sensory characteristics of food, during distribution, marketing and consumption (Sant'Ana and Araujo, 2007).

In the presence of oxygen, ironizing radiation is known to have detrimental effects on animal fats such as lipid peroxidation and rapid onset of rancidity (Molins, 2001). However, these effects can be minimized by irradiating meat or poultry in the frozen state and/or packaging under vacuum or modified atmosphere (Marapana and Wijetunga, 2009).

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CHAPTER THREE

3. EFFECT OF GAMMA IRRADIATION ON NUTRITIONAL QUALITY OF SMOKED GUINEA FOWL (*Numida meleagris*) MEAT.

3.1. INTRODUCTION

Guinea fowl (*Numida meleagris*) production is a major source of revenue to many Ghanaians with significant role in nutrition and food security (Issaka and Yeboah, 2016). The Ghanaian populace has savored delicacies from this bird due to its well-known economic and socio-cultural uses in the lives of many rural and resource-poor households, especially in the northern sector (Teye and Adam, 2000). Nutritionally, the meat of the fowl has high protein and low fat content compared to other meats, such as chicken, beef and pork (Moreki *et al.*, 2010; Warriss, 2000). Guinea fowl meat is also known to be rich in essential amino acids, fatty acids, vitamins and minerals (Tlhong, 2008).

Due to the nutritional quality of the guinea fowl meat, it has resulted in processing of the meat into shelf stable products and ready-to-eat meat products such as smoked, grilled/roasted, barbequed, dried and fried guinea fowl meat (Issaka and Yeboah, 2016; Tlhong, 2008). In most cases, the meat is re-smoked or dried to maintain its dryness as well as control mould attack and insect infestation during storage, as had been usually practiced for smoked-dried fishes in Ghana (Issaka and Yeboah, 2016, GPFO, 2003). However, the occasional re-smoking/drying of the meat affects nutritional quality, and the frequent handling and poor packaging expose the meat to microbial decomposition which pose serious threat to public food safety. The

conventional heat treatment is not an efficient method to control spoilage bacteria as had been mostly seen in chicken meat (Park *et al.*, 2010), and inadequate storage such as chilling have limited shelf-life (Sweet *et al.*, 2006). As such, post-processing methods and the use of innovative techniques in maintaining and/or improving nutritional diversity and safety of the meat for the benefit of both consumers and the meat industry need to be explored.

Processing of food by irradiation as an innovative technique, has been considered as one of the safest methods in maintaining quality and safety of meat and meat products (Artes *et al.*, 2007; Badr, 2004). Since irradiation does not considerably raise the temperature of food being processed, nutrient losses are relatively minor which are significantly less than nutrient losses related to other preservation methods such as cooking, drying and sterilization (Diehl, 1995; ICGFI, 1991). Gamma irradiation is also a well-known technology for reducing and protecting food from pathogenic microorganisms and extending shelf life without compromising nutritional properties of food products (Hajare *et al.*, 2014; Farkas, 2006).

Although, this treatment leads to some biochemical changes that could affect the nutritional adequacy of food, the inherent protective quality of the food mostly render the effects negligible (ICGFI, 1999, Giroux and Lacroix, 1998). Hence, it is essential to assess the irradiation effect on the nutritional quality of whole meat products. Since, no studies on irradiation process on the nutritional quality of guinea fowl meat has been documented, the present study aimed at determining the effect of gamma irradiation on the nutritional compositions of smoked guinea fowl meat.

3.2. MATERIALS AND METHODS

3.2.1. Rearing of birds

Helmeted guinea fowls were purchased from the commercial farm of the Livestock Production and Research Center (LIPRC) of the University of Ghana, Legon. Birds were intensively reared (raised under typical poultry intensive pen system) to 16 weeks of age before being slaughtered. They were kept in confinement under controlled environment and fed with compounded feed (complete commercial diets for broiler chickens) provided *ad libitum*.

3.2.2. Preparation of smoked Guinea fowl meat

3.2.2.1. Slaughtering and dressing

The helmeted (grey) guinea fowls were slaughtered and de-feathered using a de-feathering machine at the farms' slaughter house. Fowls were eviscerated to get rid of all entrails (Plate 3.1). Carcasses were then washed in warm clean water, drained of excess liquid, packaged in transparent polyethylene bags and stored frozen for further treatments.

Plate 3.1: Freshly dressed guinea fowl meat

3.2.2.2. Cold storage and curing

Guinea fowl carcasses were stored in a cold room/chamber (temperature of -10 ^{o}C) on the farm for a period of two weeks (14 days) before processing. The frozen carcasses were then thawed and cured in salt-sugar solution (3.5 salt: 1 sugar: 5 water) for 24 h before smoking.

3.2.2.3. Smoking

Cured guinea fowl carcasses were smoked in a smoke house for less than 24 h at 67 ± 3 ^{o}C. Meats were hung on metal holders with intensive burning of wooden planks, and simultaneously by gradual emission of smoke from Neem tree. Smoked meats (Plate 3.2) were cooled and then prepared for packaging.

Plate 3.2: Freshly smoked guinea fowl meat

3.2.2.4. Packaging

The smoked guinea fowl meats were divided into 4 groups (based on radiation treatment doses to be used), each group comprised equal sample of weight (500g ±1g). Eight (8) out of the 10 samples comprised breast portions of weight (500 g) each for sensory analysis, and the two other equal portions (125 g each, making 500 g) of thighs and drumsticks for other analysis. Meat samples were packaged in a 26.5cm×27.7cm HDPE zipper bags (Johnson Ziploc, double zipper, USA).

3.2.3. Irradiation of smoked Guinea fowl meat

Irradiation was carried out at the gamma irradiation facility at the Radiation Technology Centre (RTC) of the Biotechnology and Nuclear Agriculture Research

Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) using Cobalt-60 source (SLL-02/515, Hungary). The packaged smoked meat samples (500 g) were irradiated at ambient temperature with irradiation doses of 0, 2.5, 5 and 7.5 kGy at a dose rate of 0.74 kGy h^{-1}. The absorbed dose was determined using ethanol chlorobenzene dosimeter.

3.2.4. Determination of proximate composition of irradiated smoked guinea fowl meat

Proximate composition, namely moisture content, crude protein (as Kjeldahl nitrogen), crude fat (as extractable component in Soxhlet apparatus) and total ash of the irradiated smoked guinea fowl meat were determined according to standard procedures of Association of Official Analytical Chemists (AOAC, 2010). Carbohydrates and metabolizable energy content of the meat were calculated using difference method and Atwater factors, respectively. All results were expressed on dry matter basis.

3.2.4.1. Moisture determination

Moisture was determined by drying 5 g of meat samples to a constant weight in an oven (Gallenkamp 300 Plus, US) at 105 ^{o}C for 5 h. The weight of dried sample was subtracted from the initial (fresh) sample weight. Percent moisture was calculated using the formula:

$$\%Moisture = \frac{Weight\ of\ fresh\ sample - weight\ of\ dried\ sample}{Weight\ of\ fresh\ sample} \times 100 \ldots\ldots\ldots \textit{Equation 1}$$

3.2.4.2. Ash determination

Ash was determined by taking the weight of empty crucible (previously preconditioned for 30 min) and that of the samples before and after incineration. A 5 g sample was weighed into the crucible and ignited in a muffle furnace at 550 ^{o}C for 10 h (samples completely ash). Samples were cooled in a desiccator for about 10 min before weighing. The percent ash was calculated using the formula below:

$$\%Ash = \frac{Weight\ of\ Ash - Weight\ of\ empty\ crucible}{Weight\ of\ sample - weight\ of\ empty\ crucible} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..Equaltion\ 2$$

3.2.4.3. Protein determination

Crude protein was determined by the Kjeldahl method/procedure based on three principles: digestion, distillation and titration.

Meat samples (2 g each) were placed in four digestion tubes containing 5 g of catalyst (4.5 g K_2SO_4 + 0.5 g $CuSO_4$). Concentrated H_2SO_4 (10 ml) was added to the mixture, placed in digestion unit and digested (heated) for 8 h. Digested samples were washed with 50 ml distilled water into distillation tubes, and 80 ml of 32% NaOH transferred into the mixture (to convert NH_4^+ to NH_3). Content was distilled into 50 ml 2% Boric acid and 3 drops of screened methylene red (indicator) in a conical flask placed at the receiving end of the distillatory, for 8 min. The resulting solution (distillate) was then titrated with excess 0.1N H_2SO_4 solution (back titration). A pink colour change representing titre value was recorded. The percent nitrogen and percent protein were calculated as follows:

$$\%Nitrogen = \frac{Titre \times 0.0014}{Weight\ of\ sample} \times 100 \ldots\ldots\ldots\ldots. Equation\ 3$$

$$\%Protein = \%Nitrogen \times conversion\ factor\ (6.25) \ldots\ldots\ldots\ldots. Equation\ 4$$

3.2.4.4. Fat determination

A 2 g homogenized smoked guinea fowl meat (flesh and skin) was weighed with a filter paper, folded and placed in thimbles. The thimbles were then placed in the extractor/apparatus and 250 ml petroleum ether (solvent) was added into pre-weighed round bottom flasks (weight of empty flask). This was followed by 10 h extraction. The extracts were evaporated with a rotary evaporator (BUCHI-R-200 Rotavapor, China), and solvent distilled off. Extracts were further dried in an oven (Gallenkamp 300 Plus, USA) at 103 °C for 1 h. It was then cooled in a desiccator and weighed. The percent fat was calculated using the formula:

$$\%Fat = \frac{(Weight\ of\ flask + fat\ extract) - (weight\ of\ empty\ flask)}{Weight\ of\ sample\ taken} \times 100 \ldots\ldots\ Equation\ 5$$

3.2.4.5. Carbohydrate determination

The carbohydrate content of the smoked guinea fowl meat was determined by the difference method (Merril and Watt, 1973) as:

$$\%Carbohydrate =$$

$$(100 - [\%\text{Moisture} + \%\text{Ash} + \%\text{Fat} + \%\text{Protein}]) \ldots Equation\ 6$$

3.2.4.6. Energy value determination

Energy was determined by summing the multiplied values of protein, fat and carbohydrate with their AT WATER FACTORS (4, 9 and 4 respectively) as proposed by Osborne and Voogt (1978):

$$Energy\left(\frac{kcal}{100g}\right) =$$

$$(4 \times \%Protein) + (9 \times \%Fat) + (4 \times \%Carbohydrate) \ldots\ldots\ldots Equation\ 7$$

3.2.5. Determination of elemental/mineral composition of irradiated smoked guinea fowl meat

3.2.5.1. Sample preparation

Smoked guinea fowl thighs and drumsticks were freeze-dried for in a freeze dry/shell system (Labconco), at the Nutrition Research Centre of Radiological and Medical Sciences Research Institute (RAMSRI), GAEC. The freeze-dried samples were pulverized with a granite-stone mortar and pestle and made into pellets (Plate 3.3) using a manual hydraulic press machine (Hydraulic unit model #3512, Carver Inc. China). Elemental composition was determined using X-ray fluorescence (XRF) technique (Jenkins *et al.*, 1995).

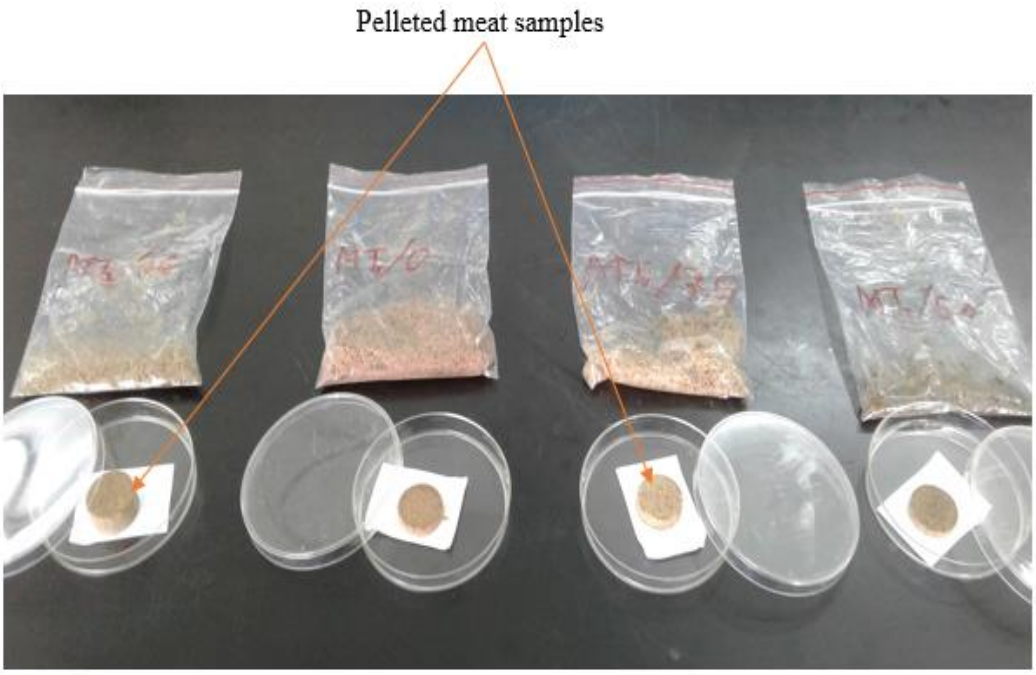

Plate 3.3: Pelleted freeze-dried smoked guinea fowl meat samples for XRF analysis

3.2.5.2. X-ray fluorescence (XRF) analysis

Sample analysis was performed using the XRF Experiment Kit (Plate 3.4) at the X-ray Fluorescence Spectrometry laboratory of the Nuclear Applications Centre of the National Nuclear Research Institute (NNRI), GAEC. Pelleted samples were placed in the sample port of the spectrometer, covered, and irradiated with a silver anode X-ray min-tube. Fluorescent photons (secondary X-rays) emanating from the meat samples were separated into spectra of characteristic X-rays energies (energy dispersive X-ray fluorescence (EDXRF), with the help of a multichannel analyzer.

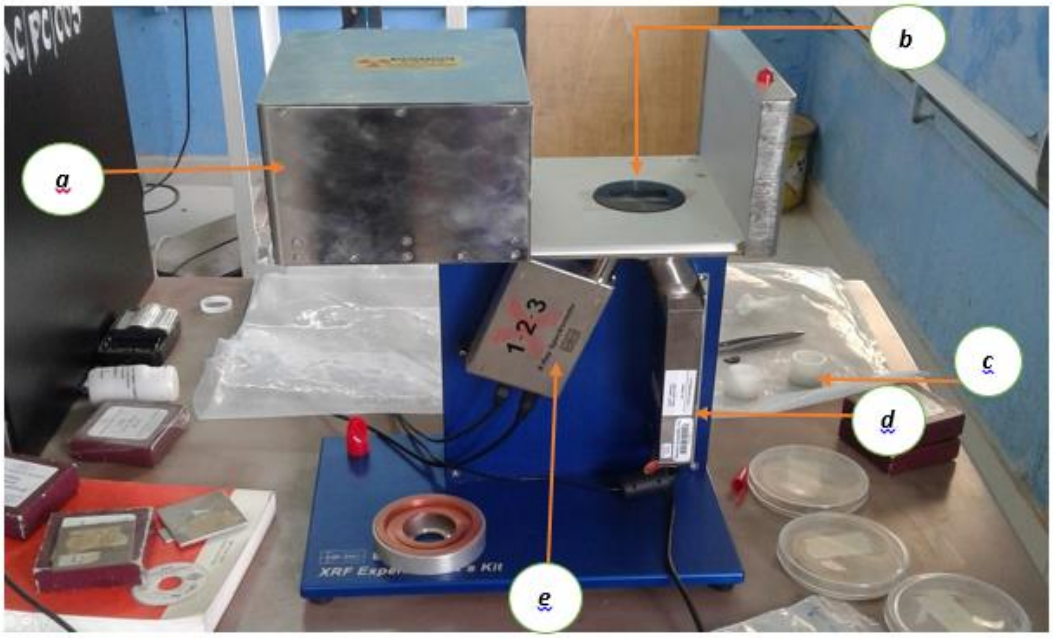

Plate 3.4: XRF Experimental kit. **a**-source casing, **b**-sample port/seat, **c**-X-ray cup with mylar, **d**-X-ray tube (source), **e**-X-ray spectrometer.

The K-series X-ray spectral lines of individual elements were cross-checked with an X-ray Line Chart (Plate 3.5) for qualitative identification of the elements present in the samples. The elements identified were then quantified.

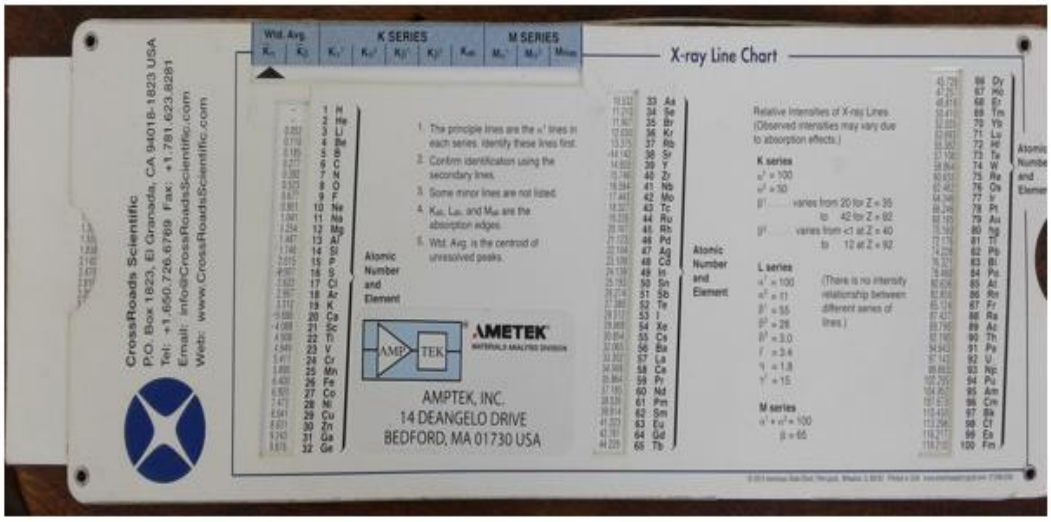

Plate 3.5: X-ray Line Chart

3.2.6. Data analysis

Proximate and mineral composition data were subjected to one-way ANOVA using StatsGraphix Centurion 16.1.11 software to distinguish significance between variables, and considered statistically significant at p value ≤ 0.05. Data were provided as mean values ± standard deviation for continuous variable, with a least significant difference (LSD) of means (5% level) for comparisons.

Energy dispersive X-ray fluorescence (EDXRF) spectrometer (Portable Amptek) was used, with standardless Fundamental Parameter (FP) approach for quantitative analysis. The concentrations of elements in the samples were determined by their calculated sensitivities. The PyMca 4.7RC6-win 32 software was used for spectrum deconvolution and computation. The output from this analysis were elements with corresponding mass fractions (percent mass), and graphical presentation of continuum, and spectrum with fitted values.

3.3. RESULTS

3.3.1. Effect of gamma irradiation on proximate composition and energy value of smoked guinea fowl meat

The effect of gamma irradiation on the proximate composition and the energy values of smoked guinea fowl meat is shown in Table 3.1. Values are presented on dry matter basis using the moisture content of the smoked guinea fowl meat. Values for proximate composition of the smoked guinea fowl meat samples ranged from 41.45 – 52.77% (Moisture), 1.83 – 2.81% (dry matter basis) (Ash), 25.86 – 43.79% (dry matter basis) (Fat), 42.12 – 53.87% (dry matter basis) (Protein) and 0.50 – 29.82% (dry matter basis) (Carbohydrate). The energy values ranged from 520.54 – 611.66 kcal/100g (dry matter basis).

In general, gamma irradiation significantly ($p \leq 0.05$) influenced the proximate composition and the energy values of the smoked guinea fowl meat. However, the observed significant effects were not dose dependent except ash content.

The moisture content values of the meat samples were 52.77% (0 kGy), 41.45% (2.5 kGy), 50.57% (5 kGy) and 48.07% (7.5 kGy). The control samples (0 kGy) recorded the highest moisture content value (52.77%) and the least value (41.45%) for 2.5 kGy treated smoked guinea fowl meat sample.

The ash content values of the meat samples were 1.83%, 2.19%, 2.51% and 2.81% for 0, 2.5, 5 and 7.5 kGy respectively. The ash value (1.83%) of the 0 kGy (control)

sample was significantly lower than values for the gamma irradiated samples. A dose dependent significant differences ($p \leq 0.05$) were observed among all the samples.

The crude protein values of the treated meat samples were 54.87% (0 kGy), 42.12 (2.5 kGy), 50.89% (5 kGy) and 44.46% (7.5 kGy). The crude protein value (53.87%) of the 0 kGy (control) sample was significantly higher than values for the gamma irradiated samples.

The fat content values for the treated guinea fowl meat were 43.79%, 25.86%, 36.59% and 29.09% for 0, 2.5, 5 and 7.5 kGy, respectively. The fat content (43.79%) of the control sample (0 kGy) significantly higher than gamma irradiated samples. Values for irradiated samples (2.5, 5 and 7.5 kGy) were however not dose dependent.

Carbohydrates values were 0.50% (0 kGy), 29.82% (2.5 kGy), 10.00% (5 kGy) and 23.64% (7.5 kGy). The value for the control sample (0 kGy) was significantly lower than the values for the irradiated samples.

The energy values of the treated smoked guinea fowl meat were 611.65 kcal/100g, 520.54 kcal/100g, 572.87 kcal/100g and 534.19 kcal/100g for 0, 2.5, 5 and 7.5 kGy respectively. Value for the control sample was significantly higher than values for the irradiated samples.

Table 3.1: Effect of gamma irradiation on the proximate composition (Dry matter basis) and energy values of smoked guinea fowl meat.

Indices	Dose (kGy)			
	0.0	**2.5**	**5.0**	**7.5**
%Moisture*	52.72±0.08^{d}	41.45±0.67^{a}	50.52±0.23^{c}	48.07±0.24^{b}
%Ash	1.83±0.04^{a}	2.19±0.11^{b}	2.51±0.12^{c}	2.81±0.01^{d}
%Fat	43.79±0.02^{d}	25.86±0.42^{a}	36.58±0.50^{c}	29.09±0.07^{b}
%Protein	53.87±0.47^{d}	42.12±0.35^{a}	50.89±0.25^{c}	44.45±0.03^{b}
%Carbohydrates	0.50±0.50^{a}	29.82±0.67^{d}	10.00±0.63^{b}	23.64±0.04^{c}
Energy (kcal/100g)	611.65±0.30^{d}	520.54±2.52^{a}	572.87±2.97^{c}	534.19±0.29^{b}

*Means ± Standard deviations with different superscripts (lower case) differ significantly ($P \leq 0.05$). *moisture value was used to calculate proximate composition on dry matter basis.*

3.3.2. Qualitative results of spectrum deconvolution and background fitting of elements in smoked guinea fowl meat.

Qualitative results from XRF mass spectrometry indicated fourteen (14) elements of corresponding energies (Fig. 3.1 and 3.2) that were compared to their K-series spectral lines (KeV) for identification. The elements with their K-series energies include: Sodium (Na-1.04), Magnesium (Mg-1.21), Aluminium (Al-1.53), Phosphorous (P-2.02), Sulphur (S-2.31), Chlorine (Cl-2.65), Potassium (K-3.31), Calcium (Ca-3.68), Scandium (Sc-4.04), Chromium (Cr-5.41), Iron (Fe-6.38), Nickel (Ni-7.40), Copper (Cu-8.03), and Zinc (Zn-8.61). Argon (Ar-2.97) is an inert gas and therefore not considered part of the minerals. Tables showing fit parameters, calibration parameters, and continuum parameters of the graphs for both irradiated and non-irradiated (control) samples are presented in Appendix 1.2.

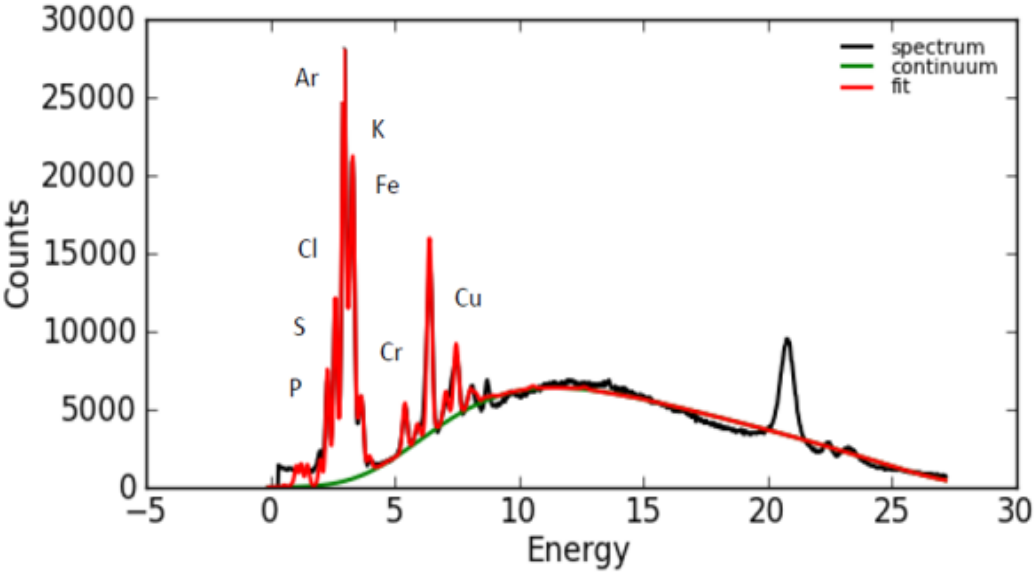

Figure 3.1: Graph of spectrum, continuum and fitted values of minerals in non-irradiated (control) smoked guinea fowl meat.

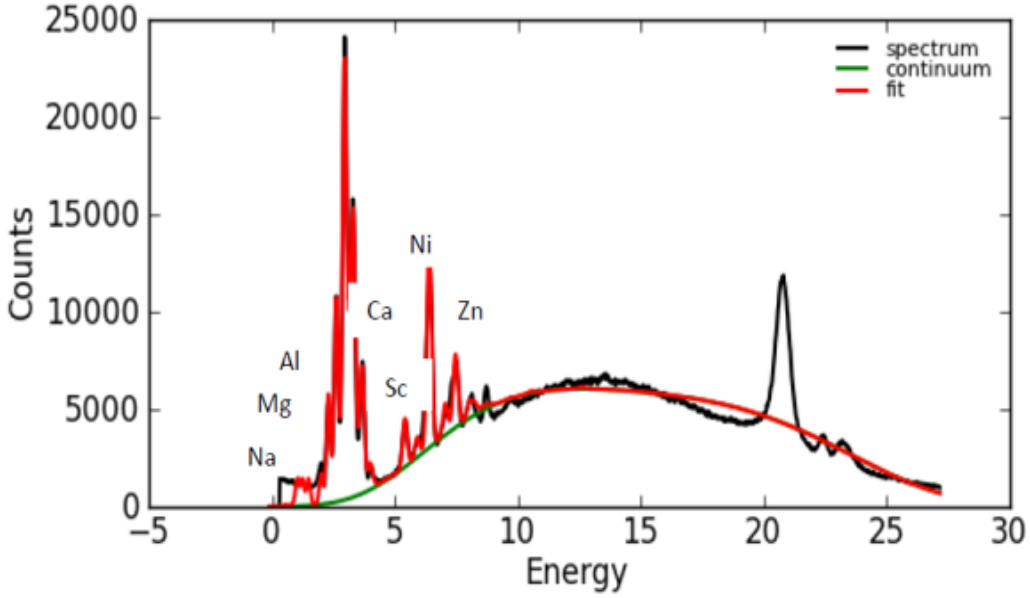

Figure 3.2: Graph of spectrum, continuum and fitted values of minerals in irradiated smoked guinea fowl meat.

3.3.3. Quantitative results of elemental concentration in smoked guinea fowl meat

Concentrations of the various elements are shown in Table 3.2. Major inorganic constituents (minerals) present in the smoked guinea fowl meat were Na (103.11 mg/kg), Mg (28.41 mg/kg), Al (11.45 mg/kg), P (2.337 mg/kg), S (4.701 mg/kg), Cl (3.499 mg/kg), K (1.275 mg/kg), and Cd (1.199 mg/kg). Na, Mg, S, and K of control samples (0 kGy) were significantly different ($p \leq 0.05$) from irradiated (2.5, 5 and 7.5 kGy) samples, with control samples of Ca being negatively significant from the irradiated samples. However, Al, P, Cl, Sc, Cr, Mn, Fe, Ni, Cu, Zn, I, and Pb were insignificantly different ($p > 0.05$) among the treated samples. Though there were some significant differences among the treated guinea fowl meat for some of the elements, in most cases increasing irradiation dose did not significantly change the mineral composition of the smoked guinea fowl meat.

Concentration of micro elements Sc, Mn, Ni, Cu, Zn, I, and Pb were so minute, and practically insignificant ($p = 0.00$) in the meat samples, as a result of their low detection limits. Three L-group elements: Cadmium (Cd), Iodine (I) and Lead (Pb), were also detected quantitatively showing their levels of concentration in the samples.

Heavy metals Mn, Cu, Zn, and Pb concentrations were approximately 0.00 mg/kg in all the samples. Fe (0.031-0.028 mg/kg), Cr (0.017-0.015 mg/kg) and Cd (1.199-1.063 mg/kg) were however, the known heavy metals present in the smoked guinea fowl meat, with Cd indicating the highest value.

Table 3.2. Effect of gamma irradiation on the mineral (elemental) composition of smoked guinea fowl meat.

Minerals (mg/kg)	**Group**	**Dose (kGy)**			
		0	2.5	5.0	7.5
Na	K	103.113±0.40^{a}	118.750±0.35^{b}	118.875±0.18^{b}	119.0161±0.02^{b}
Mg	K	28.41±0.35^{a}	29.76±0.35^{b}	29.87802±0.19^{b}	29.74604±0.37^{b}
Al	K	11.455±0.17^{a}	12.25±0.35^{a}	12.21731±0.39^{a}	12.21731±0.39^{a}
P	K	2.337±0.18^{a}	2.37±0.07^{a}	2.185391±0.33^{a}	2.120522±0.42^{a}
S	K	4.701±0.07^{b}	3.692±0.18^{a}	3.547865±0.38^{a}	3.66825±0.21^{a}
Cl	K	3.499±0.35^{a}	3.401±0.14^{a}	3.34185±0.22^{a}	3.129126±0.53^{a}
Ar	K	27.85±0.35^{b}	24.095±0.18^{a}	24.17367±0.06^{a}	23.88337±0.48^{a}
K	K	1.275±0.07^{b}	0.8978±0.07^{a}	0.8978±0.07^{a}	0.89787±0.07^{a}
Ca	K	0.0905±0.07^{a}	0.2205±0.03^{b}	0.2205±0.03^{b}	0.2205±0.03^{b}
Sc	K	0.002103±0.00^{a}	0.005562±0.00^{a}	0.005572±0.00^{a}	0.005562±0.00^{a}
Cr	K	0.017305±0.00^{a}	0.015105±0.00^{a}	0.01521±0.00^{a}	0.015105±0.00^{a}
Mn	K	0.002755±0.00^{a}	0.002708±0.00^{a}	0.002703±0.00^{a}	0.002703±0.00^{a}
Fe	K	0.03165±0.00^{a}	0.02822±0.00^{a}	0.02841±0.00^{a}	0.02841±0.00^{a}
Ni	K	0.006468±0.00^{a}	0.005512±0.00^{a}	0.005572±0.00^{a}	0.005562±0.00^{a}
Cu	K	0.001339±0.00^{a}	0.001045±0.00^{a}	0.001045±0.00^{a}	0.00103±0.00^{a}
Zn	K	0.00023±7.07E-7^{a}	0.000249±9.89E-7^{a}	0.000224±0.00^{a}	0.000249±9.89E-7^{a}
Cd	L	1.199±0.14^{a}	1.063±0.07^{a}	1.068±0.06^{a}	1.063±0.07^{a}
I	L	0.005736±0.00^{a}	0.008239±0.00^{a}	0.008129±0.00^{a}	0.008119±0.00^{a}
Pb	L	0.000177±0.00^{a}	0.00013±3.53E-7^{a}	0.00013±3.5E-7^{a}	0.00013±9.89E-7^{a}

Means ± standard deviations in the same row with different superscripts are significantly different ($p \leq 0.05$) from each other.

3.4. DISCUSSION

3.4.1. Impact of gamma radiation on the proximate composition of smoked guinea fowl (Numida meleagris) meat

In the present study, gamma irradiation was found to significantly influence the major components of the smoked guinea fowl meat (Table 3.1). The nutritional quality as measured by the total carbohydrates, fibre, protein, energy, some vitamins and minerals have been reported not to be affected by gamma irradiation (2.5, 5 and 10 kGy) (Hajare *et al.*, 2014). Also, most food macronutrients and micronutrients (vitamins and inorganic salts) are not affected by 10 kGy range ionizing dose with regard to their nutrient contents and digestibility (Mostafavi *et al.*, 2012). However, some literatures have reported some significant changes in food macronutrients with increasing irradiation doses range of 2-6 kGy (Al-Bachir, 2013; Al-Bachir and Othman, 2013).

3.4.1.1. Effect on moisture content

Gamma irradiation significantly reduced the moisture content of the smoked guinea fowl meat. The observation in the present study is in contrast with studies by Kanatt *et al.* (2015) and Al-Bachir and Zeinou (2014) who reported no significant differences in the moisture content of irradiated (2.5, 5 and 10 kGy) chicken, lamb and buffalo meat and irradiated (2, 4 and 6 kGy) goat meat respectively. Also, earlier study by Badr (2005), found that moisture content of breast and leg muscle of chicken was not affected by irradiation. The disparity in the present study could result from the meat type and processing condition. However, results are similar to that of Al-Bachir

(2013), who reported significant reduction of moisture content of irradiated (2, 4 and 6 kGy) chilled meat product (*Kubba*) compared to non-irradiated samples.

3.4.1.2. Effect on protein content

The protein content of the irradiated smoked guinea fowl meat was significantly reduced with fluctuating values from the non-irradiated samples. Recent studies by Haque *et al.* (2017), Al-Bachir and Zeinou, (2014), and Modi *et al.* (2008) have reported crude protein content of meat not significantly changed with irradiation, which contradict that of the present study. The disparity in the present study and the reported studies could be attributed to conditions used during irradiation, processing and the food type although, similar doses were used. On the other hand, low and medium doses have been reported to induce a slight breakdown of food proteins into lower molecular weight protein and amino acids (Al-Bachir, 2013) which could account for the significant changes of the protein content in the present study. Results, were however, in agreement with Al-Bachir (2013), who reported significant decrease of protein content of irradiated (2, 4 and 6 kGy) chilled meat product (*Kubba*).

3.4.1.3. Effect on lipids (fat content)

Results showed that the fat content of irradiated meat samples significantly ($p < 0.05$) decreased compared to the non-irradiated meat samples. Similar results were observed by Al-Bachir (2013), and Al-Bachir and Othman (2013) who reported a significant decrease of fat content between gamma irradiated (2, 4 and 6 kGy) and non-irradiated *Kubba* and chicken sausage respectively. The results in the present study however, contradict that of Haque *et al.* (2017), who reported a total dose-dependent increase

of ether extracted fat content in beef with increasing dose (2, 4 and 6 kGy). The authors explained that irradiation causes the degradation of large lipid molecules which in due course adds to the fat of the sample thereby increasing the fat constituent with increasing irradiation doses (Al-Bachir and Zeinou, 2014; Yilmaz and Gecgel, 2007).

3.4.1.4. Effect on ash content

Ash contents of the smoked guinea fowl meat in the present study increased with increasing irradiation dose in a dose dependent manner. The results are in agreement with that of Gecgel (2013) and Al-Bachir and Zeinou, (2014), who reported an increased ash content with increasing irradiation dose (1, 3, 5 and 7 kGy) in meatball and (2, 4, and 6 kGy) in goat meat respectively. However, results observed in the present study contradict that of Haque *et al.* (2017), who reported decreased ash content in beef with increasing irradiation doses (2, 4, and 6 kGy in beef). The disparity could result from the meat type and processing conditions (freshly processed vs. smoked).

3.4.2. Effect of gamma irradiation on the elemental composition (micronutrients) of smoked guinea fowl meat

There were no significant differences in the mineral composition of the irradiated smoked guinea fowl meats. It has been proven that irradiation, regardless of the dose, has no effect on essential minerals, in terms of either amount or bioavailability (Diehl *et al.*, 1991). Also, an evidence base for the effect of irradiation on nutritional content

of meat has been summarised and been shown that minerals are relatively unaffected by irradiation irrespective of doses applied (FDA, 1997; Diehl, 1995).

Hajare *et al.* (2014) reported on gamma irradiation doses 2.5 and 10 kGy not having effect on the microelements of nasogastric liquid feed (NGLF). Al-Bachir and Zeinou (2014) reported no effect of gamma irradiation (2, 4 and 6 kGy) on both macro and micro elements of goat meat, which agree with results reported in the present study. It could be explained that elements in a sample only get excited when irradiated, and when the source is withdrawn, the elements fall back to their ground state without affecting the nature and concentration of the elements, thus only identify and quantify the elements.

3.4.2.1. Effect on Heavy metals

Poultry meat are known to be contaminated with toxic elements such as arsenic, cadmium or lead through contact with equipment or materials on the farm, factory or movement through marketing channels (Filazi *et al.*, 2017). These three toxic elements are known to induce widespread adverse health effects (Sanap and Jain, 2015; Kurnaz and Filazi, 2011). Iron (Fe), Chromium (Cr) and Cadmium (Cd) were the known heavy metals found in the smoked guinea fowl. Results in the present study were compared to safe limits of metals concentration for human consumption.

The average Fe concentrations in the present study (≈0.03 mg/kg) were far below the safe limits of 4.49-15.0 ppm as reported by Kobia *et al.* (2016). The Fe values in the present study were also lower than values reported by Ampofo *et al.* (2017) and Kobia *et al.* (2016) in smoked game meat. The high iron content reported by these authors

were attributed to the interaction between the meats and the metal grid or iron gauze that were used to grill and smoke the meats, since the metals are often made of iron which may get deposited during the processing.

Cadmium is a metal that is prominent as an environmental contaminant resulting from natural, industrial, and agricultural sources (Filazi *et al.*, 2017). It is reported that persons who are non-smokers are exposed to Cd through foodstuffs (Akerstrom *et al.*, 2014) hence, it is not suprising to observe high levels of Cd contamination in the smoked guinea fowl meat as a result of the smoke deposited on the meat during processing. Results of Cd exceeded that of Kobia *et al.* (2016) and Imaobong (2015) who reported lower levels of 0.10 ppm and 0.024-0.17 mg/kg in grilled and smoked bush/game meat and chicken meat respectively. The concentration of Cd (1.199 mg/kg) also, exceeded the permissible limit of 0.5 ppm (0.5 mg/kg) (FAO/WHO, 2000) and 0.33 ppm as cited by Ampofo *et al.* (2017). The disparity in the present study could be attributed to the smoking process.

Cadmium has been classified as human carcinogenic (Group 1) by the International Agency on Cancer Research (IACR, 2012), and its exposure lead to increased risk for lung, endometrium, urinary bladder and breast cancer, as underlined by the European Union Food Safety Authority (EFSA, 2009). It is thus, necessary to avoid and/or reduce the bioaccumulation of such toxic element through appropriate processing technologies.

Irradiation, irrespective of the doses applied had no effect on the heavy metals although, values were lower in irradiated samples than the non-irradiated sample.

3.5. CONCLUSION

Gamma irradiation significantly affected the major nutritional components of smoked guinea fowl meat, but the effect was not dose-dependent. Mineral compositions of the meat sample were however, not significantly affected by the gamma irradiation doses. Iron, Chromium and Cadmium were the heavy metals found in the smoked guinea fowl meat. The iron and chromium contents were far below safe limit of metals concentration for human consumption however, cadmium was above safe limit. It is thus recommended that prolong smoking of poultry meat should be avoided to reduce levels of such toxic elements in the meat product. Gamma irradiation dose of 5 kGy is ideal for irradiating smoked guinea fowl meat without detrimental effect on the major food components, as values were close to that of non-irradiated samples.

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CHAPTER FOUR

4. EFFECT OF GAMMA IRRADIATION ON POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SMOKED GUINEA FOWL (*Numidia meleagris*) MEAT

4.1 INTRODUCTION

Smoked guinea fowl meat has become a favourite meat for many Ghanaian populace because of its nutritive value, low fat content, peculiar flavour, low production cost, and other important socio-cultural uses (Teye and Adam, 2000). Its diverse usefulness and importance in curbing malnutrition and as food and income security, makes it highly preferred to other meats, especially among the three northern regions of Ghana. However, the traditional mode of processing and preservation, for instance smoking exposes the meat to toxic and hazardous substances, such as polycyclic aromatic hydrocarbons (PAHs) in the environment and foods (SCF, 2002). These toxic substances (PAHs) are cancer-producing compounds which results from incomplete combustion and pyrolysis of wood and other smoke agents used in smoking meats (Hitzel *et al.*, 2013).

Polycyclic aromatic hydrocarbons (PAHs) are known to be the largest group of compounds demonstrated to be carcinogenic and mutagenic in humans (Wenzl *et al.*, 2006). Dietary intake has been the common route of human exposure to PAHs for non-smokers and non-occupationally exposed population (Phillips, 1999; Guillén *et al.*, 1997). Processing methods such as smoking, roasting, grilling and drying, among others leads to generation and increase of PAHs levels in food (Tongo *et al.*, 2017;

SCF, 2002). The presence of PAHs in food is a matter of public health concern that necessitates constant monitoring. Scientific evidence has indicated a strong association between consumption of processed meat and increased cancer risk, especially colorectal cancer (Oostindjer *et al.*, 2014). Some of the health effects associated with PAH exposure on humans include growth retardation, low birth weight, small head circumference and low IQ of children, damaged DNA in unborn children, and damaged endocrine systems (Shen *et al.*, 2008). Other disorders include skin changes as a result of dermal exposure, and reproductive-related effect such as early menopause due to destruction of ova, have also been identified with PAHs (Essumang *et al.*, 2012).

Radiation processing has become an essential technology in the food industry. It has been an effective technique in many applications of food safety such as the decontamination and inactivation of microbial organisms from poultry, meat, and their products (Thayer 1995). Gamma irradiation has been reported to be one of the most potent advanced oxidation processes (AOPs), employed for the decomposition of various pollutants such as pesticide residues (Khalil and Al-Bachir, 2017). The gamma rays have also been deployed to decompose PAHs in certain foods (Malarut and Vangnai, 2018; Khalil and Al-Bachir, 2017; Khalil *et al.*, 2016; Khalil and Al-Bachir, 2015). With ample research in the use of irradiation in the control of microbial contamination in meat, limited studies have focused on gamma irradiation effect on PAHs contaminant in meat and poultry products.

In Ghana, studies on levels, characterisation and health risk assessment of PAHs have been reported in some fish and meat products (Bandowe *et al.*, 2014; Essumang *et al.*, 2014; Abdallah, 2013; Palm *et al.*, 2011). However, little information exists on the occurrence and levels of PAHs in locally produced smoked guinea fowl meat. Also,

there is insufficient documentation on appropriate use of techniques in reducing and eliminating PAHs in foods, especially smoked poultry meat. The present study therefore, aimed at investigating the effect of gamma irradiation as a decontaminating technique on the types and levels of PAHs and their carcinogenic derivatives in smoked guinea fowl meat.

4.2 MATERIALS AND METHODS

4.2.1. Experimental sample and Sample preparation

Forty (40) helmeted guinea fowls (male and females), intensively reared (raised under typical poultry intensive pen system) to 16 weeks of age were purchased from the commercial farm of the Livestock Production and Research Center (LIPRC) of the University of Ghana. Pre-slaughtering through to smoking of the meat was performed at the farm (as described in section 3.2.2.1 – 3.2.2.3). Irradiation of the smoked meat was carried out (described in section 3.2.3) and further analysis done at the Ghana Atomic Energy Commission (GAEC) and Ghana Standards Authority (GSA).

4.2.2. Determination of PAHs as a contaminant in smoked guinea fowl

4.2.2.1. Sample preparation

In order to examine PAHs diffusion from meats' exteriors to their interiors, the smoked guinea fowl meat (thigh and drumstick) treated with and without gamma irradiation, was randomly sampled from both skin and flesh, and stored frozen for

analysis. The method used after sample preparation involved extraction, clean-up, and GC/MS analysis.

4.2.2.2. Extraction of PAHs

Polycyclic aromatic hydrocarbons (PAHs) extraction was performed by applying the organic/direct solvent extraction (DSE) technique for solid foods (Wenzl *et al.*, 2006). The meat samples were thawed, homogenized and weighed for the extraction process. Ten gram (10 g) of each sample was weighed with a balance (Mettler Toledo) into extraction flask and 50 ml of 1:1 *n*-hexane/acetone mixture was added and sonicated (Plate 4.1A) for 30 min in an ultrasonic cleaner/bath (Bransonic 220, Branson, U.S.A). After sonication, filtration with a Whatman filter paper (Whatman Int. Ltd., UK) was performed and the filtrate kept in a 250 ml round bottle flask. Extraction was carried out on each sample three times and the filtrates (extracts) combined. Sample extract was then concentrated (Plate 4.1B) using a rotary evaporator (BUCHI-R-200 Rotavapor, China). All equipment and glassware were cleansed with acetone to minimize contamination throughout the experiment.

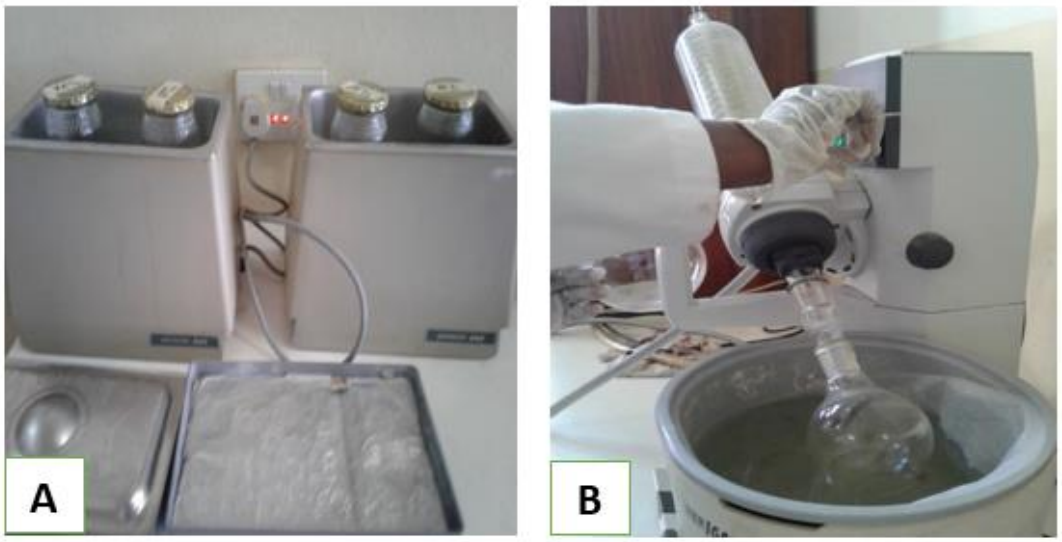

Plate 4.1: (**A**): Sonication with ultrasonic bath (**B**): Concentration of extract with rotary evaporator

4.2.2.3. Combined silica-activated charcoal clean-up of extracts

Solid phase extraction (SPE) method adopted by Jánská *et al.* (2006) was applied for sample clean-up.

Silica–activated charcoal clean up columns (Plate 4.2 A and B) were prepared by packing 4 g and 2 g of silica and anhydrous sodium sulphate respectively in the column. The packed columns were each conditioned with 10 ml 1:1 acetone/hexane after which the extracts were passed through the columns and the elutes collected into 50 ml bottles. The column was then eluted with 5 ml of 1:1 hexane/acetone. Elutes were further purified with 2 g activated charcoal (Plate 4.2 B) as an adsorbent material for removal of lipid and colouring materials observed in the samples. The cleaned elutes were concentrated to almost dryness. Sample residue was dissolved with 2 ml ethyl acetate and extract picked into sample vials using Pasteur pipette. Vials with samples were then stored refrigerated (±4 ^{o}C) and analysed with GC/MS at the Ghana Standards Authority (GSA).

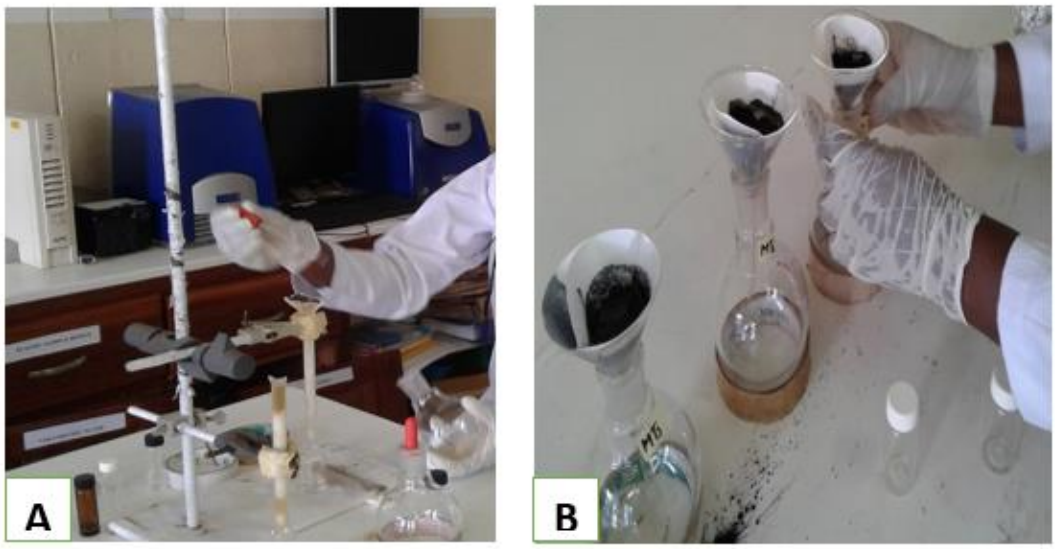

Plate 4.2: (A) Clean-up of samples with silica/sodium sulphate column, and **(B)**: Activated charcoal

4.2.2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry (GC-MS) method was used for the analysis. The GC-MS equipment used has these specifications: Instrument type: 7000C GC-MS Triple Quad (Agilent Technologies, USA); Column: BF-5MS; Carrier gas: Helium; Column flow: 1.0 mL/Min; Injection Mode: Splitless; Detector: Mass Selective Detector. The PAHs concentrations were determined using the headspace sampling technique, involving purging of samples with flow of carrier gas (solvent degassing) and analytes trapped for analysis.

Identification of PAHs was carried out by comparing their retention times with those of PAH mixed standards (Standard of 18 PAH in acetonitrile) which was used for generating calibration curves. These standard PAHs comprised: 2-Methylnaphthalene; 1-Methylnaphthalene; Naphthalene; Acenaphthylene; Acenaphthene; Fluorene; Anthracene; Phenanthrene; Fluoranthene; Pyrene; Chrysene; Benzo(a)anthracene; Benzo[k]fluoranthene; Benzo(a)pyrene; Benzo[b]fluoranthene; Benzo[g,h,i]perylene;

Dibenz[a,h]anthracene; and Indeno(1,2,3,c,d)pyrene. A solvent blank (ethyl acetate) was injected to ensure that the system was free from contaminants or interfering peaks. Summary report of quantitative analysis of the 18 non-alkylated PAHs (concentration in ppb) is presented in appendix II. Calculated concentrations were performed using the formula:

$$Actual\ PAHs\ concentration\left(\frac{\mu g}{kg}\right) = \frac{(Final\ Conc. - Blank)}{Sample\ weight} \ldots\ldots\ldots Equation\ 1$$

4.2.3. Human health risk assessment of PAHs in smoked guinea fowl meat.

Cancer risk due to dietary exposure to PAHs in the smoked guinea fowl meat was evaluated using PAH4 Index, individual 7 PAHs carcinogenic potencies (B[a]P*teq*), carcinogenic toxic equivalents (TEQs), and dietary daily intake (DDI).

4.2.3.1. PAH4 Index as a measure of carcinogenicity of PAHs

The PAH4 Index (first four PAHs known to be carcinogenic) was estimated based on review by the Panel on Contaminants in the Food Chain (CONTAM Panel) relating to occurrence and toxicity of PAHs in food (EFSA, 2008). This was calculated as the sum of the first four PAH8 (eight PAHs known to be carcinogenic), using the formula:

$$PAH4 = \sum (B[a]A) + (CHR) + (B[a]P) + (B[b]F) \ldots\ldots\ldots Equation\ 2$$

Where: B[a]A = benzo[a]anthracene; CHR = chrysene; B[a]P = benzo[a]pyrene and B[b]F = benzo[b]fluoranthene

4.2.3.2. Toxic Equivalent Factor (TEF) and Total Toxic Equivalent (TEQ)

Total toxic equivalent (TEQ) of seven PAHs mixture classified by U.S. EPA (USEPA, 1993 as reported by Bojes and Pope, 2007) as probable human carcinogens was calculated by applying toxic equivalency factors (TEFs) (Nisbet and LaGoy 1992). The TEQi (Equation 3) was calculated using the concentration of each selected PAHs and multiplied by its TEF value. Results obtained was utilized to calculate the carcinogenic toxic equivalents (TEQs) of the mixture by summing the 7 B[a]P*teq* (Meng *et al.,* 2005) (Equation 4):

$$TEQi = Ci \times TEF \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 3$$

$$\text{TEQs} = \sum_{i=1}^{q} TEQi \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 4$$

Where:

Ci = initial concentration of individual PAHs (congener *i*).

TEF = toxic equivalent factor

TEQs = total toxic equivalents of the mixture of the PAHs

TEQi = toxicity equivalency of individual PAHs

$q \in N$ = number of included PAHs with the assumed carcinogenic effect.

Evaluated TEQ values were compared with a Screening Value to assess the health risks of PAHs on people who consume meat. Screening value was calculated using equation 5 (Wu *et al.*, 2012; Cheung *et al.*, 2007):

$$SV = \frac{[(RL/CSF) * BW]}{CR} \ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 5$$

Where:

SV = screening value

RL = maximum acceptable risk level (10^{-5})

CSF = oral cancer slope factor (7.3×10^{-3} μg/kg day^{-1})

BW = average Ghanaian adult body weight (70 kg)

CR = meat consumption/ingestion rate (0.0477 kg/day)

4.2.3.3. Dietary Daily Intake of PAHs from consumption of smoked guinea fowl meat

The Dietary Daily Intake (DDI) of PAHs in the smoked meat was estimated (Equation 6). The daily intake of PAHs was evaluated by multiplying the respective PAH concentration in each meat samples by the rate of meat ingestion (IR/CR) for an average Ghanaian adult weight. Evaluation of DDI was calculated for the seven probable human carcinogenic PAHs and sum of the 16 PAHs analyzed. The DDI was calculated using the formula proposed by Halek *et al.* (2007):

$$DDI = Ci * IR \ldots\ldots\ldots\ldots\ldots Equation\ 6$$

Where:

DDI = dietary daily intake

Ci = PAHs concentration

IR = meat ingestion rate for Ghanaian adults (0.0477 kg/day)

4.2.4. Data analysis

Data for PAHs were subjected to a one-way ANOVA, distinguishing significance between groups using Microsoft Excel and StatsGraphix Centurion XVI software. Data were provided as mean values ± standard deviation for continuous variable, with a least significant difference (LSD) of means at a 95% confidence level ($p \leq 0.05$) for comparison.

4.3. RESULTS

4.3.1. Concentrations of polycyclic aromatic hydrocarbons (PAHs), total PAHs, total CPAHs and PAH4 index of smoked guinea fowl meat

The impact of gamma irradiation on concentrations of individual polycyclic aromatic hydrocarbons (PAHs), total PAHs, carcinogenic PAHs (CPAHs) and PAH4 index of the smoked guinea fowl meat is shown in Table 4.1. Out of the 18 target PAHs, 16 of EPA priority PAHs compounds were detected in the smoked guinea fowl meat samples (irradiated and un-irradiated) namely, Naphthalene (Nap); Acenaphthylene (Anl); Acenaphthene (Ane); Fluorene (Flu); Anthracene (Ant); Phenanthrene (Phen); Fluoranthene (Flt); Pyrene (Pyr); Chrysene (Chr); Benzo(a)anthracene (B[a]A); Benzo[k]fluoranthene (B[k]F); Benzo(a)pyrene (B[a]P); Benzo[b]fluoranthene (B[b]F); Benzo[g,h,i]perylene (B[g,h,i]P); Dibenz[a,h]anthracene (D[a,h]A); and Indeno(1,2,3,c,d)pyrene (I[1,2,3,c,d]P) (Table 4.1). Anthracene, was below detection limits for dose 0, 2.5 and 7.5 kGy samples.

The concentrations of the 16 EPA priority detected compounds (PAHs) ranged from 0.002 – 9.22 µg/kg (Table 4.1). Concentration of Benzo(a)pyrene (B[a]P) as a marker for carcinogenicity reported the highest value of 9.221 µg/kg for non-irradiated (control) samples (Table 4.1). The total sum of the concentrations of individual PAHs identified ranged from 10.622 – 58.366 µg/kg. Also, the total concentrations of carcinogenic PAHs (CPAH) ranged from 0.568 – 28.187 µg/kg (Table 4.1).

Table 4.1: Effect of gamma irradiation on polycyclic aromatic hydrocarbons (PAHs), total PAHs, total CPAHs and PAH4 concentrations of smoked guinea fowl meat

	Dose (kGy)			
PAHs (µg/kg)	**0.0**	**2.5**	**5.0**	**7.5**
1-Methylnaphthalene	BLD	BLD	BLD	BLD
2-Methylnaphthalene	BLD	BLD	BLD	BLD
*Acenaphthene	0.042±0.001^{b}	0.085±0.001^{c}	0.118±0.001^{d}	0.022±0.001^{a}
*Acenaphthylene	0.176±0.001^{b}	0.418±0.001^{c}	0.433±0.001^{d}	0.046±0.001^{a}
*Anthracene	BLD	BLD	0.124±0.001^{b}	BLD
***Benzo(a)anthracene	5.126±0.001^{d}	0.917±0.001^{c}	0.401±0.001^{b}	0.159±0.001^{a}
***Benzo(a)pyrene	9.221±0.001^{d}	1.054±0.001^{c}	0.057±0.001^{b}	ND
***Benzo[b]fluoranthene	7.184±0.001^{d}	1.486±0.001^{c}	0.466±0.001^{b}	0.264±0.001^{a}
**Benzo[g,h,i]perylene	5.237±0.00^{d}	1.278±0.00^{c}	0.002±0.001^{b}	ND
**Benzo[k]fluoranthene	4.491±0.001^{d}	0.745±0.001^{c}	0.177±0.001^{b}	ND
***Chrysene	6.656±0.012^{d}	1.043±0.011^{c}	0.504±0.013^{b}	0.144±0.013^{a}
**Dibenz[a,h]anthracene	5.272±0.00^{d}	1.286±0^{c}	0.002±0.001^{b}	ND
*Fluoranthene	0.367±0.001^{d}	0.106±0.001^{c}	0.084±0.001^{b}	0.031±0.001^{a}
*Fluorene	0.153±0.001^{a}	0.266±0.001^{c}	0.281±0.001^{d}	0.157±0.001^{b}
**Indeno(1,2,3,c,d)pyrene	5.268±0.00^{d}	1.285±0.00^{c}	0.002±0.001^{b}	ND
*Naphthalene	8.445±0.126^{a}	16.851±0.128^{d}	13.997±0.095^{c}	9.639±0.116^{b}
*Phenanthrene	0.358±0.001^{c}	0.087±0.001^{a}	0.402±0.001^{d}	0.126±0.001^{b}
*Pyrene	0.367±0.001^{d}	0.106±0.001^{c}	0.084±0.001^{b}	0.031±0.001^{a}
Total PAHs	**58.366**	**27.017**	**17.140**	**10.622**
CPAHs (PAH8)	**48.456**	**9.096**	**1.615**	**0.568**
PAH4	**28.187**	**4.5**	**1.428**	**0.567**
LMW	**9.176**	**17.709**	**15.358**	**9.992**
HMW	**49.190**	**9.308**	**1.782**	**0.630**
ΣPAHs	**3.242±3.320^{b}**	**1.501±3.813^{a}**	**0.952±3.213^{a}**	**0.590±2.227^{a}**

*Values are means ± standard deviations. Means in the same row with different superscripts are significantly different ($p \leq 0.05$) from each other. ND-non detected, BLD = below detection limit of GC/MS. *Non-carcinogenic PAHs, ** & ***Carcinogenic PAHs (PAH8), *** Carcinogenic PAHs used to derive PAH4 Index*

The average mean values for the samples were 3.24 (0 kGy), 1.50 (2.5 kGy), 0.95 (5 kGy) and 0.59 µg/kg (7.5 kGy) respectively. There was no statistically significant difference ($p \geq 0.05$) between the means of the irradiated samples (2.5, 5 and 7.5 kGy) however, significant differences were observed between the irradiated and non-irradiated sample means.

The PAHs detected were grouped into low molecular weight (LMW, 2-3 ring) and high molecular weight compounds (HMW, 4-7 rings). The LMW compounds (PAHs) recorded were: Nap, Anl, Ane, Flu, Phen, and Ant, having total value of 9.176 µg/kg. The HMW compounds (PAHs) recorded were B[b]F; B[k]F; B[a]P; In[1,2,3,c,d]P; D[a,h]A, Pyr, Chr, B[a]A, B[g,h,i]P, and Flt, having total PAHs value of 49.190 µg/kg in the non-irradiated meat samples. The total concentration of the LMW PAHs was lower than that for HMW PAHs. Naphthalene and B[a]P were the dominant congeners of the total PAHs and the most common LMW and HMW PAH residues respectively among the samples (Table 4.1).

Gamma irradiation significantly ($p \leq 0.05$) reduced the concentrations of individual PAHs, total PAHs, CPAH and PAH4 index in the smoked guinea fowl meat. The reductions in PAHs concentrations were dose-dependent; thus increasing gamma irradiation dose resulted in decreasing PAHs concentrations (Table 4.1). The concentrations of most PAHs of the gamma irradiated samples were significantly ($p \leq 0.05$) lower than that for the control (0 kGy) sample. Compounds, namely B[k]F, B[a]P, B[g,h,i]P, D[a,h]A, and I[1,2,3,c,d]P were not detected at 7.5 kGy of gamma irradiation (Table 4.1). There was an initial increase of Naphthalene concentration

from 8.445 (0 kGy) – 16.851 µg/kg (2.5 kGy) and then a significant decrease with increasing irradiation dose.

4.3.2. Effect of gamma irradiation on Toxic Equivalency Factor and Total Toxic Equivalent of PAHs

Table 4.2 represents the toxic equivalent factors (TEFs) and B[a]P*teq* (TEQi) for the individual 7 PAHs probable human carcinogens. The toxicity equivalents (TEQs) of individual 7 PAHs in the 0 kGy smoked guinea fowl meat were B[a]P (0.513 µg/kg), CHR (0.067 µg/kg), B[a]P (9.221 µg/kg), B[b]F (0.718 µg/kg), B[k]F (0.449 µg/kg), D[a,h]A (5.272 µg/kg), and B[g,h,i]P (0.052 µg/kg) (Table 4.2). The concentrations of the individual calculated TEQi were normalized into percentages as shown in Figure 4.1 for easy trend analysis.

Table 4.2: Toxic equivalent factors (TEFs) and B[a]P*teq* of seven probable carcinogenic PAHs in smoked guinea fowl meat.

PAHs (µg/kg)	TEFs*	Dose (kGy)			
		0	2.5	5.0	7.5
Benzo(a)anthracene	0.1	0.513	0.092	0.040	0.016
Chrysene	0.01	0.067	0.010	0.005	0.001
Benzo(a)pyrene	1	9.221	1.055	0.057	ND
Benzo[b]fluoranthene	0.1	0.718	0.149	0.047	0.026
Benzo[k]fluoranthene	0.1	0.449	0.075	0.018	ND
Dibenz[a,h]anthracene	1	5.272	1.286	0.003	ND
Benzo[g,h,i]pyrene	0.01	0.052	0.013	0.000	ND
TEQs		**16.292**	**2.679**	**0.169**	**0.044**

TEFs - Nisbet and LaGoy (1992), ND = not detected*

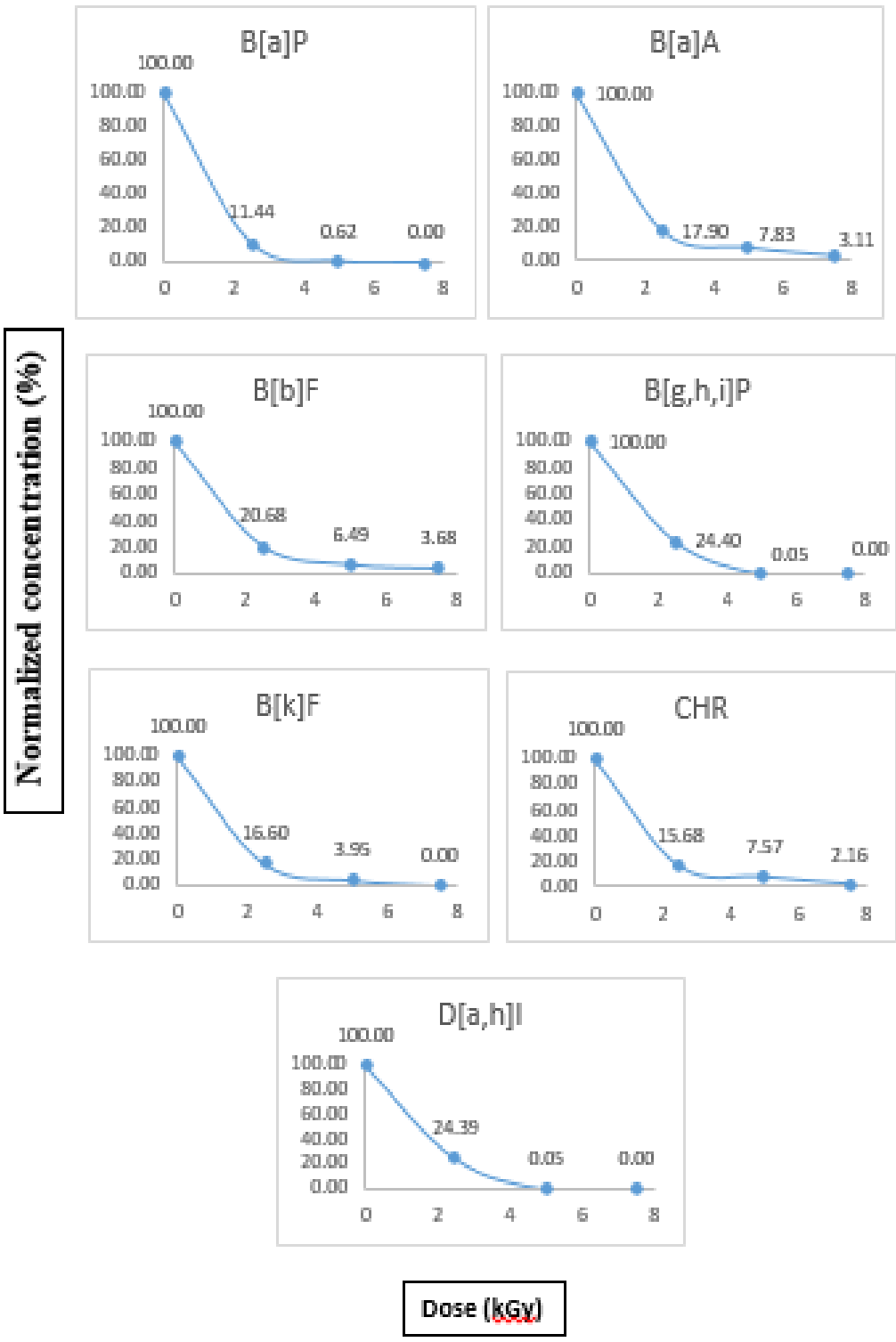

Figure 4.1: Individual toxicity equivalents (TEQi) of seven probable carcinogenic PAHs in smoked guinea fowl meat.

The individual calculated TEQi decreased exponentially with increasing gamma irradiation dose (Fig. 4.1). Similarly, the total carcinogenic toxicity equivalents (TEQs) of the mixture of selected PAHs decreased exponentially with increasing gamma irradiation dose (Figure 4.2). This exponential decrease in PAHs correlated perfectly (R^2 = 0.98) with increasing gamma irradiation dose (Fig. 4.2). The exponential equation obtained for the above trend was $y = 100.41e^{-0.82x}$, where y = TEQs (%) and x = gamma irradiation dose (kGy). The decrease in total toxic equivalents (TEQs) of PAHs follows the order: TEQ (0) > TEQ (2.5) > TEQ (5.0) > TEQ (7.5).

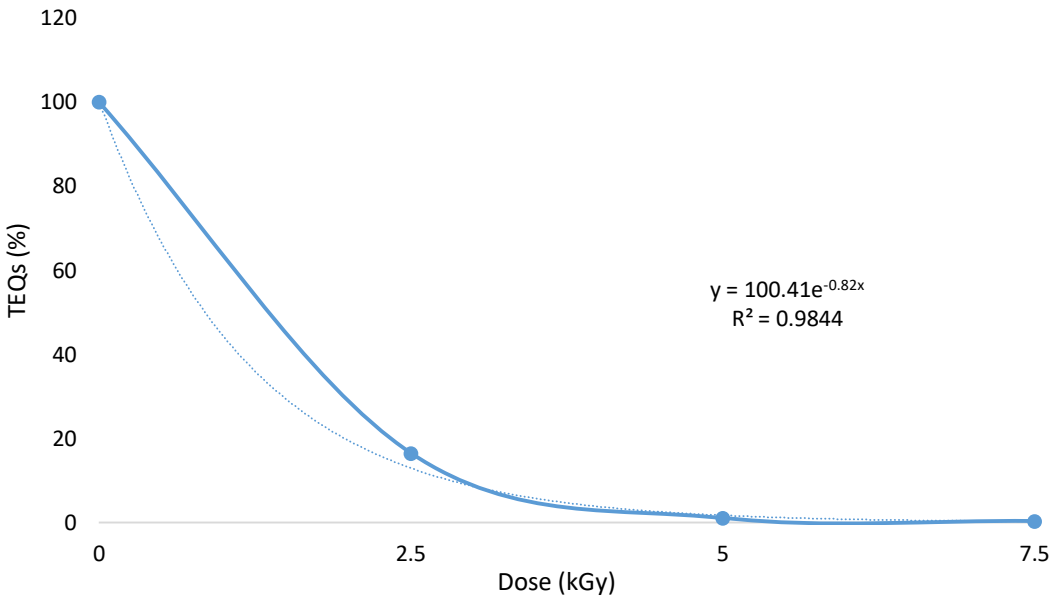

Figure 4.2: Total toxicity equivalency (TEQs) of 7 PAHs at gamma irradiation doses

4.3.3. Estimated screening values (SV) and Daily Dietary Intake (DDI) values

4.3.3.1. Screening value (SV)

An estimated screening value (SV) of 20.103 µg/kg/day was obtained. The value, was above the TEQs of all treated samples (16.292, 2.679, 0.169 and 0.044 µg/kg) for the seven probable human carcinogenic PAHs.

$$SV = \frac{[(10^{-5}/7.3\times10^{-3})\times70]}{0.0477}$$

$$= 20.103 \text{ µg/kg/day}$$

4.3.3.2. Daily Dietary Intake (DDI)

The estimated DDI values (Table 4.3) for total PAHs were 2.784, 1.289, 0.818 and 0.507 µg/day for dose 0, 2.5, 5.0 and 7.5 kGy samples, respectively. The highest value of DDI was recorded for control samples (0 kGy), and this value decreased substantially with increasing irradiation dose (Table 4.3). Dietary daily intake for individual and sum of seven probable human carcinogens also recorded highest for control sample (2.060 µg/day).

Table 4.3: Dietary daily intake (DDI) of the seven probable human carcinogens and sum of 16PAHs in smoked guinea fowl meat

DDI (µg/day)	Dose (kGy)			
	0.0	**2.5**	**5.0**	**7.5**
Benzo(a)anthracene	0.245	0.044	0.019	0.008
Chrysene	0.318	0.050	0.024	0.007
Benzo(a)pyrene	0.440	0.050	0.003	0.000
Benzo[b]fluoranthene	0.343	0.071	0.022	0.013
Benzo[k]fluoranthene	0.214	0.036	0.008	0.000
Dibenz[a,h]anthracene	0.251	0.061	0.000	0.000
Benzo[g,h,i]pyrene	0.250	0.061	0.000	0.000
Total	**2.060**	**0.373**	**0.077**	**0.027**
Σ16PAHs	**2.784**	**1.289**	**0.818**	**0.507**

4.4. DISCUSSION

4.4.1. Effect of gamma irradiation on concentration of PAHs in smoked guinea fowl meat

The present study recorded concentrations for both low and high molecular weight PAHs in smoked guinea fowl meat samples (Table 4.1). The observed PAHs were similar to those reported by Roseiro *et al.* (2011) for Portuguese traditional dry/fermented sausage. However, the concentrations of the PAHs recorded in the present study were lower (58.366 µg/kg) than values of 3237.10 and 1702.85 µg/kg reported by Roseiro *et al.* (2011) for traditional processing and modified industrial processing of Portuguese traditional meat product, respectively. This disparity could be attributed to various factors, such as the wood type, temperature the wood attains

during combustion, moisture content of the wood, concentration of oxygen, and the ventilator velocity in the combustion chamber as well as the fat content of food (Škaljac *et al.,* 2014; Hitzel *et al.,* 2013; Guillen *et al.,* 2000).

Burning of wood has been shown to produce large amounts of PAHs (Stolyhwo and Sikorski, 2005). An ideal, commercial heat source for smoking meat products globally is the Beech (*Fagus sylvatica*) woodchips, as they produce good quality smoke with highly acceptable sensory attributes (Hitzel *et al.,* 2013). Neem tree (*Azadirachta indica*), a common deciduous hardwood tree, is known to exhibit similar qualities of Beech with lower levels of PAH4 (Malarut and Vangnai, 2018). Neem tree has been established as the most feasible and low cost alternative to Beech woodchips for application in the smoking industry, hence, its application in the present study.

Research has shown that the quantity of PAHs formed during pyrolysis and pyrosynthesis increases with increase in smoking temperature and duration (Simko, 2002; Chen and Chen, 2001). In the present study, smoking was done for 17 h at 67 ± 3 ^{o}C using Neem tree.

Gomes *et al.* (2013) reported a decline in light PAHs levels in meat products with low fat, and concluded that fat content is the second most important factor influencing PAHs levels in food. Nakamura *et al.* (2008) also reported similar factor in model dimmers relating fat content to the increase and differences obtained in PAHs concentration as a result of the pyrolysis of fat occurring in meat when food is in direct contact with a flame. Alternatively, the melted fat from the meat or fish dripping onto

the heat source generates PAHs which is deposited on the meat surface as the smoke rises (SCF, 2002; Philips, 1999).

The LMW (2-3 rings) and HMW PAHs (4 rings and above) was based on their total number of aromatic rings as classified by Ferrarese *et al.* (2008). That is, the medium molecular weight (MMW) compounds (Pyrene and Fluoranthene) made up of 4 rings were added to the HMW compounds (5-7 rings). These MMW compounds had been classified by Palm *et al.* (2011) and ATSDR (1995).

The results obtained for the LMW PAHs in the present study were in disparity to reported values by Roseiro *et al.* (2011) and Pagliuca *et al.* (2003), who reported much higher levels of LMW PAHs in smoked meat and meat smoked with deciduous trees (hard wood), respectively. Also, the concentration of LMW PAHs in the present study disagreed with published data on smoked and grilled meats (Alomirah *et al.*, 2011; Farhadian *et al.*, 2011; Farhadian *et al.*, 2010; Stumpe-Viksna *et al.*, 2008). These LMW compounds have been reported to have low toxicity profile (non-carcinogenic PAHs) compared to the HMW compounds on the EPA list (Kumar *et al.*, 2016), hence are considered safe. The significance of the LMW PAHs for dietary intake has also been confirmed by the study on the dietary exposure to PAHs in Spain (Marti-Cid *et al.*, 2008).

The higher concentrations of HMW PAHs in the control samples could be attributed to the fact that the HMW PAHs are more resistant to degradation both in the meat sample and the environment, as stated by Ongwech *et al.* (2013), who reported similar

trend in smoked fish (*Lates niloticus*). These authors explained that the probability of LMW PAHs being converted to HMW compounds is high, through the addition of pyrolytic products from prolonged smoking/wood combustion. Moreover, pyrolysis of these aromatic hydrocarbon residues has been reported to result in the formation of additional HMW PAHs, subsequently increasing their concentrations (Guillen and Sopelana, 2004; Simko, 2002; Guill**é**n *et al.*, 1997). A similar profile was also reported by Palm *et al.* (2011). These authors reported higher concentrations of HMW than LMW PAHs in smoked fish from Ghana. The authors attributed the difference to residues of previous pyrolytic processes that may have occurred in the smoking chamber, and the additional pyrolytic products from wood combustion during re-smoking to the intact PAH molecules forming HMW PAHs.

In the present study, Naphthalene (Nap) was detected as the most dominant congener of the total PAHs. This observation agreed well with findings of Tongo *et al.* (2017). Also, Gomes *et al.* (2013) and Roseiro *et al.* (2011) reported Nap as the most common light PAH occurring in some smoked meat and meat products. However, other studies have reported Phenanthrene as the most common light PAH in smoked meat (Malarut and Vangnai, 2018; Purcaro *et al.*, 2009). The concentrations of Nap increased after the smoked guinea fowl meat was exposed to gamma irradiation. However, the increase was not dose dependent. Butt and Qureshi (2008), Popov and Getoff (2005) and Cooper *et al.* (2002) stated that increase in concentrations of Nap with irradiation could emanate from the effect of a degradation/grouping of event of another PAHs compound, such as Fluorene which is the main resultant compound of radiolytic degradation of Fluoranthene. Conversely, this LMW compound is non-carcinogenic and occurs abundantly in nature. Taking into account the properties of

Nap, FAO/WHO (1991) cited by Abdallah (2013) stated that the EU has recommended Naphthalene content in smoked meat to be as low as reasonably achievable (ALARA).

The presence of Anthracene in the 5 kGy sample could possibly have come from residual contamination of other hydrocarbons from different samples that were run along with the guinea fowl analytes.

In general, gamma irradiation significantly decreased the concentrations of the detected PAHs (Table 4.1) in the present study. These decreases were found to be exponential with increasing irradiation dose (Fig. 4.1). Similar trends have been reported by Khalil *et al.* (2016) in wheat grains. The reductions in the concentrations of PAHs could be attributed to radiation depolymerisation. As stated earlier, the concentrations of LMW PAHs were lower than those for HMW in control samples; however their concentrations in the irradiated samples were much higher than those for HMW PAHs. The increases in concentrations of LMW PAHs and with subsequent decreases in concentrations of HMW PAHs after gamma irradiation could be inferred from their interaction with gamma rays which readily breakdown the 5 benzenic rings, reducing their concentration drastically (Khalil *et al.*, 2016). These observations agreed with findings of Khalil *et al.* (2016), who stated that HMW aromatic compounds degenerated into LMW aromatic compounds by gamma irradiation thus, increasing the concentration of the LMW PAHs. The new LMW compounds formed therefore, become less resistant to natural/biological decomposition process and subsequently, become less hazardous for human health (Butt *et al.*, 2005).

4.4.1.1. Total 16 PAHs of control and irradiated smoked guinea fowl meat

Results from the present study demonstrated that degradation of the 16 PAHs increased along with the increasing applied dose by 53.71%, 70.63% and 81.80% respectively, compared with the control sample. Thus, a total reduction of 81.80% was obtained at 7.5 kGy. Khalil and Al-Bachir (2017) reported similar PAHs reduction in pea seeds treated with different doses of gamma irradiation. The authors reported a total reduction of about 77% for 5 kGy and 96% at the highest dose of 15 kGy. A decrease in PAHs concentration by 70% for doses higher than 5 kGy in wheat grains have also been reported by Khalil *et al.* (2016).

4.4.1.2. Total carcinogenic compounds (ΣCPAH) of control and irradiated smoked guinea fowl meat

European Food Safety Authority (EFSA, 2008) reported the use of PAH8 (ΣCPAH), just as PAH4 to be more suitable indicators for the occurrence and toxicity of PAHs. However, the authority stated further that PAH4 should be preferred to PAH8, as the former provides a good estimate of the carcinogenic potency of the PAHs detected. The value reported for non-irradiated (control) PAH8 samples (48.456 µg/kg) was however, higher than values reported by Alomirah *et al.* (2011) in whole grilled chicken (20.3 µg/kg), and Ongwech *et al.* (2013) in smoked fish sampled from different markets (37.18, 21.17 and 28.65 µg/kg). All PAH8 compounds were significantly ($p \leq 0.05$) reduced with increasing gamma irradiation dose.

4.4.1.3. Benzo[a]pyrene (B[a]P) concentrations of control and irradiated smoked guinea fowl meat

Concentration of B[a]P, has been accepted as a marker for the occurrence and effect of carcinogenic PAHs in smoked foods as specified in the European Commission (EC) Regulation No 1881/2006 (EC, 2006). Benzo[a]pyrene is known to be carcinogenic to humans – group 1 (IARC, 2010) hence, its reduction and/or elimination in food is required. The highest value of B[a]P in the control (0 kGy) sample (9.221 µg/kg) was above the acceptable limit of 5 µg/kg (EU Commission Regulation, 2014), but values for irradiated meat samples were all below the limit. The B[a]P of irradiated samples reduced drastically; a reduction percentage of 88.5, 99.38 and 100% for 2.5, 5.0 and 7.5 kGy, respectively. Khalil *et al.* (2016) also observed ~50 % reduction of B[a]P in wheat kernels irradiated with gamma rays. Conversely, the maximum levels have been lowered to 2.0 µg/kg as from 2014/2015 by the EU Regulation (2014). However, the initial maximum level of 5 µg/kg is maintained as the standard level in smoked fish and meat in Ghana. Data based on PAHs risk characterization has proved B[a]P not to be considered as the only satisfactory indicator for the occurrence or magnitude contamination by carcinogenic PAHs in food products, but the use of PAH4 marker is more applicable (EFSA, 2008).

4.4.2. Carcinogenic human health Risk assessment of PAHs in smoked guinea fowl meat

4.4.2.1. Carcinogenic potencies (B[a]Pteq) and toxic equivalents (TEQ) of PAHs in the control and irradiated smoked guinea fowl meat

There were significant differences ($P \leq 0.05$) in B[a]P*teq* values among the irradiation samples and the control. The TEQi (Table 4.2) for the individual 7 PAHs were all below the maximum risk limit for B[a]P (5 μg/kg), except values for D[a,h]A and B[a]P which recorded 5.272 and 9.221 μg/kg respectively for the control samples. Gamma irradiation significantly reduced the TEQs of the 7 PAHs.

The observed decrease in the TEQs of the individual 7 PAHs concentration and their mixture (Fig. 4.1 and 4.2), varied according to PAHs kinds. These phenomena have also been reported by other authors (Khalil and Al-Bachir, 2017; Khalil *et al.*, 2016; Khalil and Al-Bachir, 2015). The decrease in TEQs values with increase in irradiation dose demonstrates the relationship between PAHs concentration and the increasing doses of gamma irradiation. This suggests that gamma irradiation could be a potent technique in decontaminating PAHs in smoked meat. It could also be inferred from the results that non-irradiated (control) smoked guinea fowl meat had higher potential to cause carcinogenic risk from consumption than irradiated samples. This could be linked to the factors as explained earlier.

4.4.2.2. PAH4 Index of the control and irradiated smoked guinea fowl meat

The PAH4 index assessment was based on the review by the Contaminants in the Food Chain (CONTAM) Panel in 2008, that PAH4 is a more suitable indicator of PAHs in food (EFSA, 2008). Based on their conclusion, new maximum levels for PAH4 were introduced whilst maintaining a separate one for B[a]P (5 µg/kg). In the present study, the estimated PAH4 index (Table 4.1) in all samples (both irradiated and non-irradiated) were below the maximum permissible level of 30 µg/kg for the sum of PAH4 in traditionally smoked meat and meat products, as recommended by Regulation (EU) No 835/2011 amended Regulation (EC) No 1881/2006 (EC, 2011; EC, 2006) and Miculis *et al.* (2011). However, new maximum levels (12 µg/kg) for the sum of PAH4 had been introduced by the EU Commission Regulation, No 1327/2014, (EU Commission Regulation, 2014). This approach was aimed at ensuring that PAHs levels in food are kept at levels that do not cause health concerns. Concentrations of PAH4 in irradiated samples (4.50, 1.428 and 0.567 µg/kg) were however, below the new regulation level of 12 µg/kg, signifying the need for this technology to be used in reducing such PAHs.

4.4.2.3. Screening value of PAHs in the control and irradiated smoked guinea fowl meat

The screening value (SV), as reported by Wu *et al.* (2012), Cheung *et al.* (2007) and USEPA (2000), is the threshold concentration of chemicals in edible tissue that is of potential public health concern. The average adult body weight and meat consumption rate among Ghanaian populace were suggested as 70 kg (Tongo *et al.*, 2015) and 0.0477 kg/day (equivalent to 17.43 kg per year), respectively, as reported by FAO

(2017). The oral slope factor (7.3 µg/g/day) and the maximum acceptable risk level (RL) value of 10^{-5} (Tongo *et al.*, 2015; USEPA, 2000; USEPA, 1993) was used for calculating the SV. Results obtained in the present study showed that the TEQ values for all the samples were below the SV of 20.103 µg/kg/day, indicating a lesser potential health effects. The results agreed well with values reported by other researchers for fish (Tongo *et al.*, 2017; Patrolecco *et al.*, 2010; Cheung *et al.*, 2007).

4.4.3. Human health risk assessment

4.4.3.1. The Dietary Daily Intake (DDI) of PAHs from consumption of non-irradiated (control) and irradiated smoked guinea fowl meat

Human intake models applied in assessing human health risks from exposure to PAHs through consumption of smoked guinea fowl meat were body weight and meat consumption rate. Consumption rate for meat in Ghana for an average adult populace of 17.43 kg per capita per year (equivalent to 0.0477 kg/capita/day) was obtained from data of the Food and Agriculture Organisation (FAO, 2017). In Ghana, smoked meat products are usually consumed as a ready to eat form of meat product (RTE meat), especially at densely populated meat consumption areas (mostly the northern populace). Hence, the concept of DDI use to assess the health risk of toxicants is vital.

Most estimated DDI values have been reported for fish species in various countries (Bandowe *et al.*, 2014; Dhananjayan and Muralidharan, 2012; Falco *et al.*, 2005; Saeed *et al.*, 1995), but limited data is provided for meat samples. The total dietary intakes (16PAHs) for the average Ghanaian adult obtained in the present study were

far lower than that of Alomirah *et al.* (2011) in smoked and grilled meats. Also, values were lower than reported values for fish from studies in Ghana (Bandowe *et al.*, 2014).

The DDI's for individual 7 probable human carcinogenic PAHs concentrations were also compared to available reference dose (USEPA, 1993) in order to determine the long-term risk associated with exposure to PAHs residues through consumption of the smoked meat. Generally, the observed DDI values in the present study were all below the reference dose. However, it should be noted that reference dose values used were for smoked fish species, since no reference dose data was found for smoked meat.

4.5. CONCLUSION

Sixteen (16) priority EPA PAHs were detected in locally produced smoked guinea fowl meat consisting of varying amounts of both LMW and HMW PAHs. Benzo[a]pyrene (9.221 µg/kg) was the highest concentration of HMW PAHs which was above tolerable risk levels of 5 µg/kg in non-irradiated meat samples, whilst Naphthalene was the highest LMW PAH. The PAH4 index was however, within the maximum acceptable risk limits of 30 µg/kg in all treated meat samples. The total PAHs concentrations and their carcinogenic derivatives were all decreased exponentially with the increasing irradiation dose. The results obtained in the present study therefore, will enhance the utilization of gamma irradiation as a potential technique for decreasing the harmful effect of carcinogenic PAHs in smoked meat and other foods.

4.6. REFERENCES

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CHAPTER FIVE

5. EFFECT OF GAMMA IRRADIATION ON THE SHELF LIFE OF SMOKED GUINEA FOWL (*Numida meleagris*) MEAT

5.1. INTRODUCTION

Guinea fowl meat has become a relishing meat in the diet of many health conscious individuals and for varying economic and socio-cultural use of the rural and peri-urban communities in Ghana, particularly, the northern sector of the country (Teye and Adam, 2000). Guinea fowl meat is an excellent source of protein for humans as well as nutrient for the growth of both spoilage and pathogenic microbes (Adzitey *et al.,* 2015). Smoking is one of the processing techniques used in extending shelf-life of guinea fowl meat. However, smoked guinea fowl meat has been found to be contaminated with microorganisms (Adzitey *et al.,* 2015). These authors have reported the presence of bacteria species, namely *Streptococcus* spp., *Proteus* spp., *Staphylococcus* spp., *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp. and *Escherichia coli* in some smoked guinea fowl meat samples sold in the Bolgatanga Municipality, Ghana (Adzitey *et al.,* 2015). Due to the unique flavour and nutritional benefits of smoked guinea fowl meat, there is a need to adopt an innovative technology in addition to smoking, to maintain and enhance the shelf life and safety status of guinea fowl meat. One of such technologies is the use of gamma irradiation.

Gamma irradiation of food has been successful, not only in ensuring food safety, but in extending shelf life of meat and poultry products due to its effectiveness in inactivating pathogens without deteriorating product quality (Mahapatra *et al.,* 2005). It is a safe technology for eradicating pathogens from raw and processed meat products

for shelf life enhancement (Kong *et al.*, 2017; Alfaia *et al.*, 2007). Irradiation at a dose of up to 10 kGy has been used in animal products as an effective, safe and economical method of food preservation, posing no nutritional, toxicological or microbiological problems (O'Bryan *et al.*, 2008; WHO, 1999).

Irradiation alone may not always be sufficient in achieving the intended effect. The dose required may produce undesirable sensory and chemical changes in some foods (Mahapatra *et al.*, 2005). Although, irradiation up to 10 kGy have been generally known to result in no change in the nutritional properties or safety of food (WHO, 1999), other studies have reported that irradiation can influence the acceleration of lipid oxidation, discoloration, and the decline of sensory properties associated with the formation of off-flavour in meat and meat products (Du *et al.*, 2002; Jo *et al.*, 1999). The above problems are essential factors which directly affect the quality characteristics of meat products.

Combination of other conventional treatments and irradiation may however, achieve desirable results. These treatments will reduce the irradiation dose required, thereby reducing the cost and preserving the quality of the product (Lung *et al.*, 2015). Also, irradiation with other conventional food preservation techniques such as heating and smoking often have synergistic antimicrobial effects, and inhibit the development of undesirable sensory and some chemical changes in food (Kim *et al.*, 2014; Marapana and Wijetunga, 2009). The use of low dose of e-beam irradiation and vacuum packaging has been reported to improve safety and shelf life of smoked duck meat (An *et al.*, 2017). However, there is little information on the impact of gamma irradiation on the shelf life of smoked guinea fowl meat.

The objective of the present study was to determine the effect of gamma irradiation on some quality characteristics of smoked guinea fowl meat during refrigeration storage. In achieving this, the microbiological, physicochemical and sensory shelf life studies were investigated.

5.2. MATERIALS AND METHODS

5.2.1. Study area

The shelf life study was conducted at the Radiation Technology Centre of the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC). Physicochemical, microbial growth and sensory (organoleptic) changes of smoked guinea fowl meat treated with different doses of gamma radiation were studied during refrigerated (3±1 ^{o}C) storage period of seven weeks.

5.2.2. Sample preparation

Forty (40) smoked guinea fowl meat, (processed as described in section 4.2.2) were collected from the farm to the laboratory for the shelf life study. Thigh and drumstick cuts from whole smoked meat were randomly apportioned into groups for microbiological and physicochemical analysis. Breast portions of the meat were used for sensory evaluation. The various meat portions were then irradiated (as described in session 3.2.2.) at different doses (0, 2.5, 5 and 7.5 kGy) and stored at refrigeration temperature (3±1 ^{o}C) for shelf life study.

5.2.3. Experimental design

A Factorial experimental design representing four doses (0, 2.5, 5 and 7.5 kGy), and four storage times (0, 2, 5, and 7 weeks) at storage temperature of 3 ^{o}C ±1 in triplicate, were used for the shelf life study. Sixty four (64) thigh and drumstick pieces (randomly grouped according to radiation treatments) were assigned for microbiological and physicochemical analysis. For sensory assessment, 40 breast meat portions (grouped into 4 sections) were randomly allocated to the dose treatments and evaluated for two (2) months.

5.2.4. Microbiological analysis

5.2.4.1. Enumeration of microorganisms

Standard pour plate count technique was used for evaluating total viable count (TVC), *Staphylococcus aureus, Escherichia coli, Salmonella* spp. and *Bacillus cereus* during the shelf life study. This was followed by isolation and identification of the presumptive microorganisms.

5.2.4.2. Culture technique

Cultures were made by plating out the meat samples onto Nutrient Agar for the prospective organisms, using a sterile inoculating loop. Ten gram (10 g) of each sample was aseptically weighed with an electronic balance (Mettler Toledo, Switzerland) in a 90 ml diluent (0.1% peptone + 0.5% NaCl), and shaken. Serial dilutions were prepared and 1 ml aliquots from each serial dilution (prepared up to

10^5) was dispensed into sterile Petri dishes. About 15 ml of molten agar was added and mixed thoroughly by rotating plates clockwise and anticlockwise. Plates were allowed to solidify and incubated at 37 °C for 48 h.

Staphylococcus aureus was estimated on Baired-Parker (BP) agar (OXOID, CM275), *E. coli* was estimated on Eosine Methlyne Blue (EMB) agar (OXOID, CM0069), *Bacillus cereus* estimated on *Bacillus cereus* (BC) agar (OXOID, CM617), and total viable count on Plate Count Agar (PCA) medium (OXOID, CM0325) (Prakash *et al.*, 2014; ISO, 2003a).

Detection and enumeration of *Salmonella* spp. (25 g samples) were also done with Xylose Lysine Deoxycholate (XLD) agar (OXOID, CM0469) for the first and last week, using the horizontal method.

All media were prepared in accordance with the Oxoid manual. Colony forming units per gram (cfu/g) of individual plates (between 30-300 colonies) were counted using a colony counter (Stuart Scientific, UK). Pure cultures were sent to the Bacteriology laboratory of Noguchi Memorial Institute for Medical Research (NMIMR) of the University of Ghana, Legon for identification of the presumptive organisms.

5.2.4.3. Purification and identification of isolates

Isolates of interest (*Staphylococcus sp.*, *E. coli*, and *Bacillus sp.*) were purified by subculturing onto fresh media (Blood Agar and Nutrient Agar), and identified using Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique (Plate 5.2). This method is capable of absolutely identifying about 4,613 different oval and non-oval microbial species based on mass

spectra of the bacterial protein profiles, including many dark pigmented, gram-negative, anaerobic rods of subgingival origin (Cabrera, 2015).

5.2.4.3.1. Identification of isolates

Using a sterile wooden toothpick, a single colony of each test sample/isolate (presumptive *E. coli, Staphylococcus* sp. and *Bacillus* sp.) was picked and direct-spotted (Schmitt *et al.*, 2013) as a thin film onto an individual circular spot surface of a polished steel MALDI-TOF MS target plate (Plate 5.1). The target plate was allowed to dry at ambient temperature (27±2 ^{o} C).

Plate 5.1: MALDI-TOF MS target plate

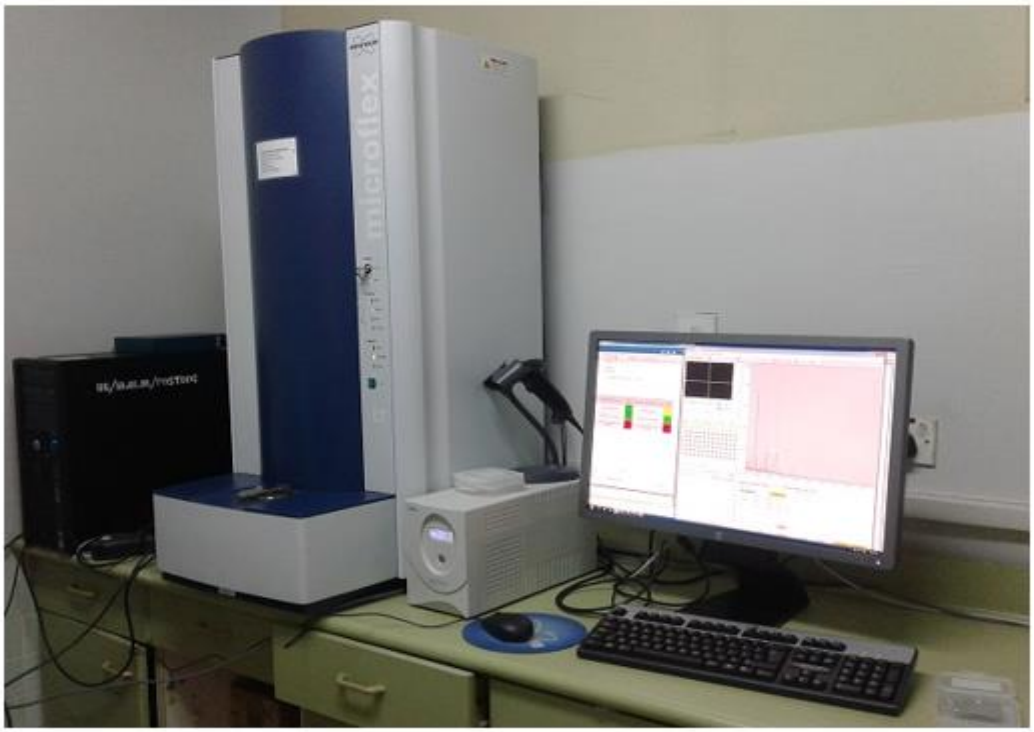

Plate 5.2: MALDI-TOF mass spectrometry

5.2.4.3.2. Formic acid extraction method

Deionized water (300 µL) was pipetted into an Eppendorf tube. One single colony was picked from the plate into the tube using a 10 µL sterile inoculating loop, and mixed thoroughly by vortexing. Ethanol (EtOH) of 900 µL was added, and mixed thoroughly. The tubes were centrifuged at a maximum speed of 14,000 rpm for 2 min, and supernatant decanted. Centrifuging was repeated and all residual EtOH was removed by pipetting it off to waste without disturbing the pellet. The EtOH-pellet was allowed to dry at ambient temperature (27±2 º C) for 2 min. A 70% formic acid (40 µL) was added to the pellet and mixed very well by vortexing and pipetting. Pure acetone (ACN) was added and mixed carefully by vortexing (same volume as formic acid – 40 µL). A 1.0 µL of supernatant was pipetted onto the MALDI-TOF target plate and allowed to air dry over the colony smears to facilitate on-plate extraction of bacterial cell proteins (Hsu and Burnham, 2014) at ambient temperature (27±2 º C).

Each spot was then subjected to a second overlay solution with 1.0 µL of MALDI matrix HCCA (10 mg/ml in 70% CH_3CN/0.1% TFA) which had been prepared following manufacturer's instructions (Bruker Daltonik, Billerica, MA, USA) and allowed to dry at ambient temperature (27±2 ^{o} C). Negative control spots on the target plate were left blank or contained only the dried matrix solution without any bacterial specimen. The whole extraction procedure was carried out within 1 h, as required.

5.2.4.4. Data processing and analysis of isolates

Data were processed and analysed with MALDI-TOF mass spectrometry analysis software (Plate 5.2) with a bench top Bruker FlexControl (Microflex LT, Bruker Daltonics, 202, Germany). Each mass spectra from the bacterial isolates were analyzed and compared with MALDI Biotyper 3.1 software (Bruker Daltonics) database (MBT 6903 MSP Library), which comprised of 4,970 distinct bacterial species, to determine the most likely microbial genus and species identification. The MALDI Biotyper log score, generated as a level of identification probability by the software of ≥ 1.7 was utilized as a threshold for reliable species identification, as endorsed for assessment of anaerobic bacteria (Hsu and Burnham, 2014). Log scores ≥ 2.0 were considered more definitive species identification, whereas log scores < 1.7 (most likely bacterial species) was considered to provide less reliable bacterial identification.

5.2.5. Physicochemical analysis

The following physicochemical properties of the meat samples namely, pH, acid value, and total acidity were analysed based on the official AOAC methods of analysis

(AOAC, 2010). The analysis were done for a period of 7 weeks (thus, weeks 0, 2, 5 and 7) in triplicates.

5.2.5.1. pH determination

In food quality, pH influences the ability of microorganisms to grow in a specific food, and is defined as the negative log (base 10) of the hydrogen ion concentration (Tyl and Sadler, 2017). The pH of the control and irradiated smoked guinea fowl meat samples was measured using a portable digital pH meter (350 pH meter, Jenway Co., England). Meat samples were cut into small pieces and homogenized using a laboratory blender. Ten grams (10 g) of homogenized samples were accurately weighed using a weighing balance (Acculab Sartorius, China). A 100 ml of water was added to the 10 g samples in 4 different labelled flasks. The solutions were shaken with a mechanical shaker (Junior Orbit Shaker, U.S.A) for 30 min. Solutions were allowed to settle and decanted into new flasks. The pH meter with electrode was inserted into each filtrate, value recorded, and the electrode rinsed with distilled water after each measurement. The pH meter was standardized against buffers of pH 4.0 and pH 7.0 standard solutions prior to determination of pH of the samples.

5.2.5.2. Total titratable acidity (TA)

Total Titratable acidity (TA) measures total acid concentration in the food samples. This concept better predicts the influence of flavour in food than pH, and is expressed in terms of predominant organic acid in the food (Tyl and Sadler, 2017). It is determined by neutralizing the acid in known weight of the food sample with a

standard base, using a pH-sensitive indicator. The volume of the titrant used together with the normality of the base and the weight of the sample is then used to calculate the titratable acidity (Tyl and Sadler, 2017).

The titratable acidity of the meat, calculated as a percentage of acetic acid equivalent (Nollet and Toldra, 2008) was measured using the filtrate (10 g of sample in 100 ml of water) previously used for pH determination (section 5.2.5.1). Three (3) drops of indicator (1% phenolphthalein) was added to the filtrate and titrated with 0.1M NaOH till a faint pink colour persisted within 15 s. Titre value was read and recorded. Titratable acidity was calculated as:

$$TA\ (\%) = \frac{V \times M\ \times\ Meq.}{(W) \times 1000} \times 100 \ldots\ldots\ldots\ldots Equation\ 1$$

Where:

V= volume of titrant, NaOH (ml)

M= Mormality of titrant (0.1M NaOH)

Meq. = Milliequivalent factor (60.05mg/g)

W= weight of sample (g)

TA= Titratable acidity

5.2.5.3. Acid value determination

Acid value test measures free fatty acids as an indication of hydrolytic rancidity (Gheisari, 2011). It is the number of milligrams of sodium (or potassium) hydroxide necessary to neutralize free fatty acids present in 1 g of fat. Free acids extracted from the meat sample was determined by titrimetric determination according to AOAC (2010).

One gram (1 g) of each meat sample was weighed in conical flasks with 25 ml of 99.8% (w/w) ethanol, and shaken for 30 min. The solution was then decanted and the filtrate used for titration. One milliliter (1 ml) of phenolphthalein (indicator) was added to the filtrate and titrated against 0.1N NaOH to light pink colour change. Titre value was read and recorded. The acid value was calculated using the formula:

$$Acid\ value\ \left(\frac{mg}{g}\right) = \frac{(V \times N) \times 56.1}{W} \ldots\ldots\ldots\ldots Equation\ 2$$

Where:

V = volume of NaOH (ml)

N = normality of NaOH (0.1N)

W = weight of sample used (g)

5.2.6. Sensory evaluation

5.2.6.1. Sample preparation

Sensory analysis was conducted at the sensory laboratory of the Radiation Technology Centre of the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC). Meat samples were divided into four portions with the two upper halved breast selected for sensory evaluation. Equal weights of the meat (500g ±1) were packaged in 26.5cm×27.7cm HDPE zipper bags (Johnson Ziploc double zipper, USA), and irradiated according to treatment process. Samples were then stored in a refrigerator at 3 ^{o}C ±1 and assessed on two occasions (before and after storage).

5.2.6.2. Consumer acceptance

Meat samples were cut into small pieces, warmed and randomly served to 20 consumer (untrained) panels, comprising staff of the Commission. Panelists used sensory evaluation booths for the evaluation. At each storage period, samples as indicated by the experimental design (dose/storage) were cut into equal sizes (about 2cm×5cm cubes) and served on 3-digit coded plastic containers for sensory evaluation. Smoked meats were judged for colour, aroma, tenderness (texture), taste and overall acceptability.

The Hedonic rating test (affective test) comprising a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) (Yoon *et al.,* 2012) was used for consumer preference and acceptability test. Structured questionnaires (Appendix 5) were used to determine the effect of irradiation doses on stated parameters. Results were recorded

in designated forms with descriptive terms, based on the organoleptic characteristics of quality deterioration (taste, aroma, colour and texture).

5.2.7. Data analysis

Microbial counts were expressed as logarithm colony forming units (CFUs) per gram (log_{10} cfu/g). The mean value ($log_{10}(x)$ and standard deviation (SD) were calculated on the assumption of a log normal distribution. Colony isolated from each medium was identified and tabulated by MALDI-TOF MS Biotyper (Microflex LT, Bruker Daltonics 202 Germany).

All data results were subjected to a Factorial ANOVA using STATISTICA 8.0 software package (StatSoft. Inc. USA). Fisher least significant difference (LSD at $p \leq 0.05$) was used for mean comparison.

5.3. RESULTS

5.3.1. Effect of irradiation on microbial load in smoked guinea fowl meat under refrigeration storage condition

The combined effect of gamma irradiation and storage period on total viable count (TVC), *Salmonella* spp. and the presumptive microorganisms (*S. aureus, E. coli,* and *B. cereus*) are shown in Table 5.1. Generally, dose-dependent significant differences ($p \leq 0.05$) were observed for all microorganisms throughout the refrigerated storage.

The total viable counts (TVC) of the smoked guinea fowl meat samples were 7.23 $\log_{10}$ cfu/g (0 kGy), 5.00 $\log_{10}$ cfu/g (2.5 kGy), 3.84 $\log_{10}$ cfu/g (5 kGy) and 2.67 $\log_{10}$ cfu/g (7.5 kGy). The TVC of the control sample (0 kGy) was reduced by 1.56 log cycle at the 7 weeks storage period. Gamma irradiation (2.5, 5, and 7.5 kGy) reduced the TVC by 2.23, 3.39 and 4.56 log cycle, respectively. The TVC for the irradiated meat samples reduced by 2.83, 2.22, and 1.38 log cycle for 2.5, 5 and 7.5 kGy, respectively over the 7 weeks refrigerated storage period.

Staphylococcus aureus counts were 3.95 $\log_{10}$ cfu/g (0 kGy), 2.79 $\log_{10}$ cfu/g (2.5 kGy), 1.03 $\log_{10}$ cfu/g (5 kGy) and below detection limit (< 1.00) for 7.5 kGy sample. Radiation dose of 2.5 kGy reduced the *S. aureus* population by 1.16 log cycle. Also, 2.5 kGy dose reduced *S. aureus* from 2.79 log cycle to undetectable levels (<1.00) by week 7. Samples treated with 5 kGy have *S. aureus* counts reduced from 1.03 log cycle to undetectable levels (1.00) at week 2. No significant differences were observed at the initial storage period (0-2 weeks) for 0 kGy sample, but there were significant

reductions afterwards during the storage period. Significant differences occurred among all irradiated samples during storage.

Esherichia coli counts were 2.21 $\log_{10}$ cfu/g (0 kGy), 1.49 $\log_{10}$ cfu/g (2.5 kGy), and below detectable limit (<1.00) for 5 and 7.5 kGy samples. The population of *E. coli* for control (0 kGy) samples was reduced by 0.15 log cycle at the 7 weeks of storage. No significant differences ($p > 0.05$) were observed among 0 and 2.5 kGy samples throughout the storage period. *E. coli* counts for 2.5 kGy sample reduced from 1.49 log cycles to undetectable levels (< 1.00) at week 7.

The counts for *Bacillus cereus* were 3.98 $\log_{10}$ cfu/g (0 kGy), 2.61 $\log_{10}$ cfu/g (2.5 kGy), 1.26 $\log_{10}$ cfu/g (5 kGy) and below detection limit (< 1.00) for 7.5 kGy. *B. cereus* in 0 kGy samples was reduced to 1.90 log cycles at the end of the refrigerated storage period (week 7). Irradiation dose (2.5 kGy) reduced the *B. cereus* by 1.34 log cycles. The 2.5 kGy sample was below detection limit at the last storage period (week 7). *B. cereus* counts for samples treated with 5 kGy reduced from 1.26 log cycles to limit below detection (< 1.00) at week 2. *B. cereus* counts for all the samples were significantly ($p \leq 0.05$) reduced during the storage period.

Salmonella, was not detected in all the meat samples (0, 2.5, 5 and 7.5 kGy) at the beginning and end of refrigerated storage period.

Table 5.1: Effect of irradiation on microbial load of smoked guinea fowl meat stored at ± 3 °C.

Organisms	Dose (kGy)	Storage (Weeks)			
		0	2	5	7
Total viable count	**0**	7.23 ± 0.01^{m}	6.15 ± 0.14^{l}	5.93 ± 0.06^{k}	5.67 ± 0.06^{j}
	2.5	5.00 ± 0.01^{i}	3.08 ± 0.06^{g}	2.85 ± 0.02^{f}	2.17 ± 0.16^{d}
	5	3.84 ± 0.01^{h}	2.17 ± 0.16^{d}	1.90 ± 0.05^{c}	1.62 ± 0.13^{b}
	7.5	2.67 ± 0.05^{e}	1.82 ± 0.07^{c}	1.59 ± 0.11^{b}	1.29 ± 0.04^{a}
S. aureus	**0**	3.95 ± 0.01^{g}	3.84 ± 0.05^{g}	2.78 ± 0.03^{f}	2.42 ± 0.09^{e}
	2.5	2.79 ± 0.01^{f}	2.09 ± 0.09^{d}	1.45 ± 0.08^{c}	$<1.00^{b}$
	5	1.03 ± 0.05^{b}	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$
	7.5	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$
E. coli	**0**	2.21 ± 0.36^{d}	2.26 ± 0.20^{d}	2.09 ± 0.09^{d}	2.06 ± 0.07^{d}
	2.5	1.49 ± 0.19^{c}	1.39 ± 0.02^{c}	1.31 ± 0.01^{c}	$<1.00^{b}$
	5	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$
	7.5	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$
B. cereus	**0**	3.98 ± 0.02^{g}	2.79 ± 0.01^{f}	2.12 ± 0.13^{e}	2.05 ± 0.09^{de}
	2.5	2.61 ± 0.49^{f}	1.68 ± 0.14^{cd}	1.32 ± 0.27^{bc}	$<1.00^{b}$
	5	1.26 ± 0.16^{bc}	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$
	7.5	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$	$<1.00^{a}$
Salmonella spp.	**0-7.5**	ND	ND	ND	ND

Mean count [log_{10} cfu/g], (n=3), detection limit = 1.00. Mean ± SD with different superscript vary significantly at $P \leq 0.05$. ND = not detected

5.3.1.1. Identification of presumptive microorganisms by MALDI-TOF mass spectrometry

The MALDI Biotyper software identified the presumptive microorganisms for most likely species and genus. The reliable species identification (with strain type) ≥ 1.7 were *Serratia marcescens* (DSM 12483) and *Staphylococcus aureus* (DSM 203IT) (Table 5.2). Score value below 1.7 was reported as no possible organism(s) identifiable hence, considered less reliable bacterial identification. However, the best

match of organisms with log scores < 1.7 were strains of *Enterobacter cloacae* (DSM 30060).

Table 5.2. Summary of identifiable microorganisms by the MALDI-Biotyper software.

Sample ID	Sample Name	Organism (best match)	Score Value
E11 (+++) (A)	RED (standard)	*Serratia marcescens*	**2.06**
E12 (+++) (B)	GOLD (standard)	*Staphylococcus aureus*	**2.26**
F7 (-) (C)	CREAM (standard)	No Organism Identification Possible	**1.57**

A=Red, B=Gold, C=Cream (colours of the isolates), E11, E12 and E7 are sample spot positions on the MALDI-TOF target plate, (+++/green colour) = passed, (-/red colour) = not passed

5.3.2. Physicochemical properties of irradiated smoked guinea fowl meat stored at refrigeration condition

The pH, acid value, total titratable acidity of irradiated smoked guinea fowl meat stored during 7 weeks (54 days) refrigerated period is shown in Figures 5.3, 5.4 and 5.5, respectively.

5.3.2.1. pH of irradiated smoked guinea fowl meat during refrigerated storage

There was no dose-dependent effect on the pH of the samples irrespective of storage week and doses applied. Generally, there was an increase in the pH of samples during the storage period. However, the pH values were within the slightly neutral region, except for 2.5 kGy sample (Fig. 5.3).

The pH values of irradiated and non-irradiated smoked guinea fowl meat ranged from 6.99 – 7.30. The least pH value (6.99) was observed for samples treated with 5 kGy at week 0, whilst the highest (7.30) was obtained for 2.5 kGy sample at the end of storage period (week 7).

Significant differences ($p \leq 0.05$) were observed in pH among the treated samples throughout the storage period. However, there were no significant differences ($p > 0.05$) between 2.5 kGy and 5.0 kGy sample at the 5^{th} week and between 0 and 7.5 kGy at week 0. Also, there was no significant difference in pH for 7.5 kGy sample at week 0 and week 7.

The control (0 kGy) and 7.5 kGy samples followed similar trends of initial decrease in pH from week 0 to week 5 and then increased at week 7. The 2.5 kGy sample declined initially at week 2 and then increased afterwards. However, 5 kGy sample did not follow the above trend, as there was a steady pH increase throughout the storage period.

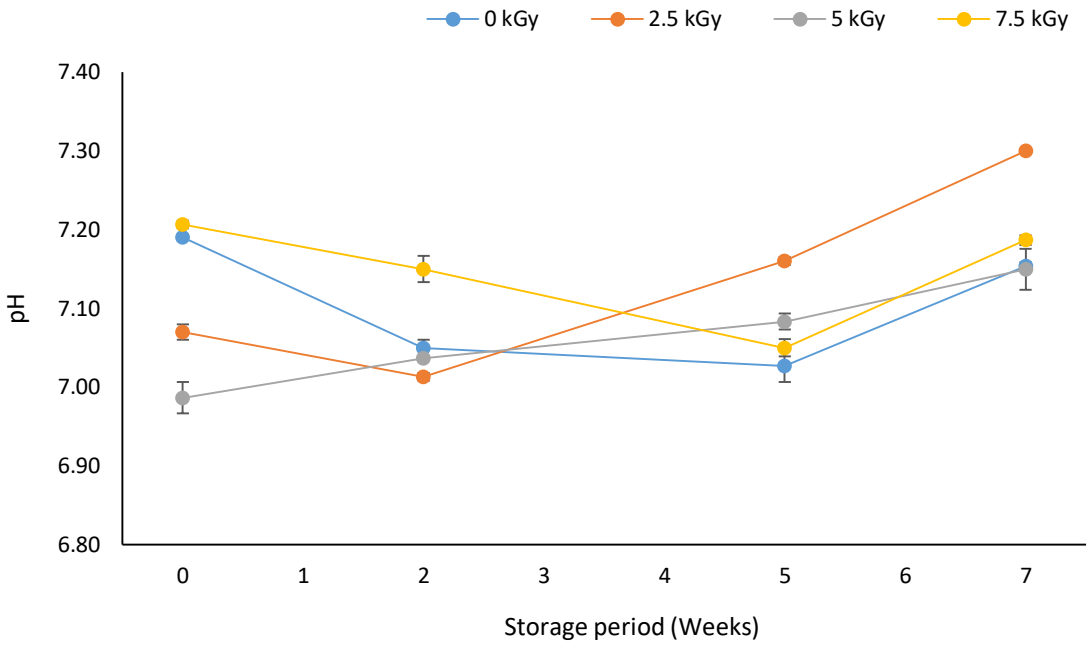

Figure 5.1: pH of irradiated smoked guinea fowl over 7 weeks storage period

5.3.2.2. Total titratable acidity of irradiated smoked guinea fowl meat during refrigeration storage condition

Total titratable acidity (TTA) of irradiated smoked guinea fowl meat over 7 weeks refrigerated storage period is presented in Figure 5.4. The TTA ranged from 0.333 – 0.610 % acetic acid. The least TTA value was observed in sample treated with 5.0 kGy dose at week 0, whereas the highest value was reported for control (0 kGy) sample at the last week of storage (week 7). Generally, there was an initial decrease of TTA at week 2, then increased steadily with increasing storage period among all samples except 5.0 kGy sample which observed a steady increase throughout the storage period.

Significant differences ($p < 0.05$) were observed among all the samples for week 0, 5 and 7. However, no significant differences ($p > 0.05$) were observed among the irradiated samples (2.5, 5 and 7.5 kGy) at week 2, but TTA for the irradiated samples were significantly different from the non-irradiated sample (0 kGy).

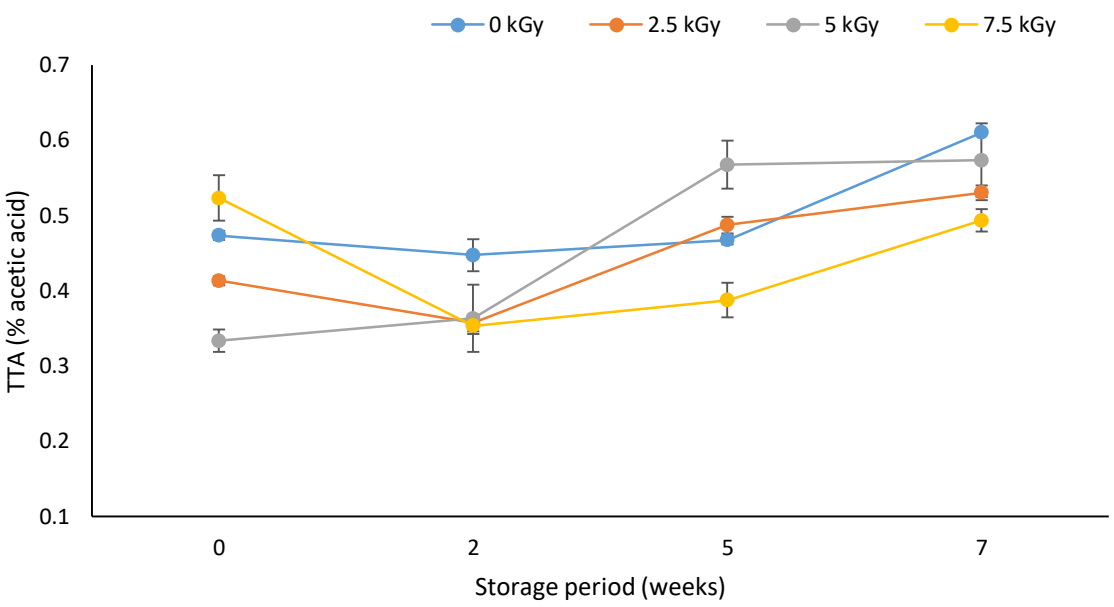

Figure 5.2: Total acidity of irradiated smoked guinea fowl meat at refrigeration storage

5.3.2.3. Acid value (AV) of irradiated smoked guinea fowl meat during refrigeration storage condition

The acid value of smoked guinea fowl meat treated with gamma irradiation and stored at refrigeration temperature is shown in Figure 5.5. Significant differences ($p \leq 0.05$) were observed among all samples throughout the storage period. Acid value of control samples during storage ranged from 7.106 – 9.35 mg/g. The least value (7.106 mg/g)

occurred at week 5 whilst the highest value was observed at week 7. Values for irradiated (2.5, 5.0, and 7.5 kGy) meat samples during storage ranged from 5.236 – 9.911 mg/g, with 7.5 kG recording the least value (5.236 mg/g) at week 2. The highest value (9.911 mg/g) was recorded by 5 kGy sample at the end of the storage period.

The acid value for the control sample decreased steadily with storage weeks and increased during the last storage period. Samples treated with 5 kGy and 7.5 kGy decreased at week 2, but increased afterwards throughout the storage period. However, the acid value of samples treated with 2.5 kGy did not follow the above trends, but rather increased steadily throughout the storage period.

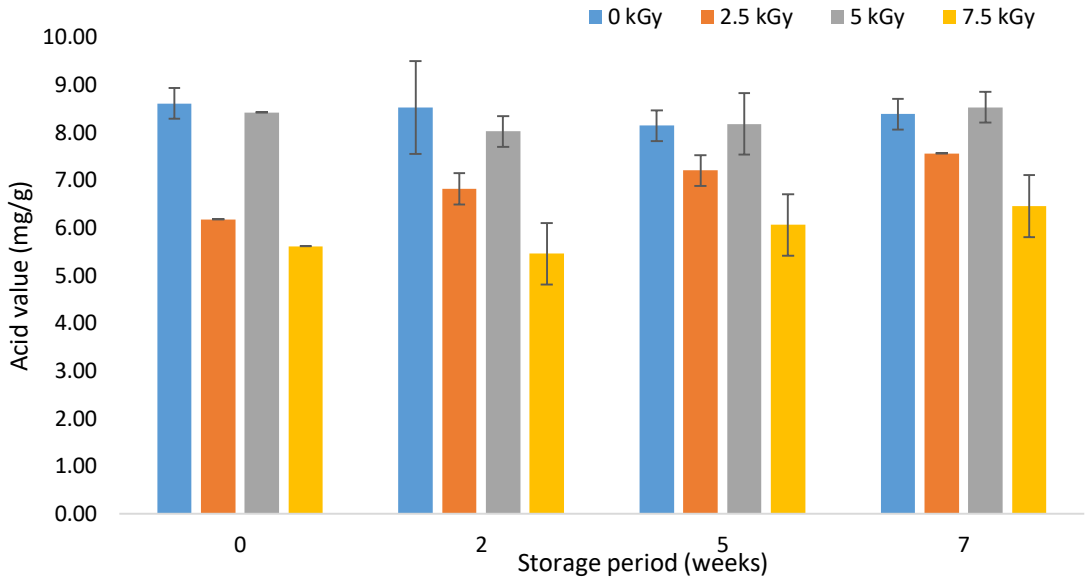

Figure 5.3: Acid value of irradiated smoked guinea fowl meat at refrigeration storage

5.3.2.4. Relationship between physicochemical parameters (acid value, pH and titratable acidity) and dose

Table 5.3 shows Pearson product moment correlations between each pair of variables (acid value, titratable acidity, pH and dose). These correlation coefficients ranged between -1 and +1 and measured the strength of the linear relationship between the variables. A strongly positive correlation (0.934) between acid value and titratable acidity was observed. A negative correlation was found between radiation dose and acid value (-0.67), as well as dose and titratable acidity (-0.447). Also, a strongly negative correlation occurred between pH and acid value (-0.814), and pH and titratable acidity (-0.857).

Table 5.3: Correlation between acid value, titratable acidity, pH and dose of irradiated smoked guinea fowl meat at 3 ± 1 °C.

Parameters	***Acid value***	***Titratable acidity***	***pH***	***Dose***
Acid value	1			
Titratable acidity	0.9338 (0.0662)	1		
pH	-0.8139 (0.1861)	-0.8571 (0.1429)	1	
Dose	-0.6702 (0.3298)	-0.4472 (0.5528)	0.1278 (0.8722)	1

Values in parenthesis ( ) are P-value. P-values below 0.05 indicate statistically significant non-zero correlations at 95.0% confidence level.

5.3.3. Sensory evaluation of irradiated smoked guinea fowl meat during refrigeration storage condition

The effect of irradiation on the sensory attributes (aroma, colour, tenderness, taste and overall acceptability) of the smoked guinea fowl meat during refrigeration storage is shown in Table 5.4. In general, there were no significant differences ($p > 0.05$) in all attributes (aroma, colour, tenderness, taste and overall acceptability) among all the samples assessed at the beginning of the storage period (Month 0). At the end of storage period, there were no significant differences ($p > 0.05$) in aroma, colour, tenderness and overall acceptability for all the samples, however some levels of significant differences were observed in taste among the samples.

The taste for all the samples (0, 2.5, 5.0 and 7.5 kGy) at month 0 was equally liked however; samples irradiated at 7.5 kGy were more preferred.

Aroma of all the samples was equally liked however, samples irradiated at 7.5 kGy had the highest score value of 7.05 and the least value of 5.85 recorded for 5 kGy at month 1.

The colour of all treated samples was equally liked; however colour for 7.5 kGy sample at month 1 was moderately liked, but liked slightly at the end of the storage period. The colour for 7.5 kGy meat sample decreased significantly with storage period.

The tenderness/texture for all samples was equally liked during the storage period. Sample irradiated at 7.5 kGy and stored at month 0 was liked moderately.

For overall acceptability, sample treated with 7.5 kGy was liked moderately at the initial month compared with other samples, whereas control sample (0 kGy) was rated higher than other samples at end of storage period.

Table 5.4 Mean preference scores of selected sensory attributes of smoked irradiated guinea fowl meat stored at 3 ± 1 °C.

ATTRIBUTES	DOSE (kGy)	STORAGE PERIOD (Months)	
		Month 0	Month 1
AROMA	0	6.25±2.15^{ab}	6.40±1.60^{ab}
	2.5	6.55±1.54^{ab}	6.75±1.33^{ab}
	5	5.85±2.37^{a}	6.05±1.23^{ab}
	7.5	7.05±1.57^{b}	6.60±1.27^{ab}
COLOUR	0	6.65±1.69^{ab}	6.20±1.45^{a}
	2.5	6.75±1.37^{ab}	6.05±1.43^{a}
	5	6.45±2.06^{ab}	6.35±2.16^{ab}
	7.5	7.25±0.97^{b}	5.75±1.37^{a}
TENDERNESS	0	6.55±1.85^{a}	6.65±1.35^{a}
	2.5	6.45±1.85^{a}	6.45±1.60^{a}
	5	6.55±2.01^{a}	6.30±1.56^{a}
	7.5	7.20±1.39^{a}	6.65±1.66^{a}
TASTE	0	6.50±2.35^{abc}	7.40±1.05^{b}
	2.5	6.95±1.43^{abc}	7.00±1.15^{ab}
	5	6.75±1.55^{abc}	5.95±1.88^{c}
	7.5	7.30±1.49^{ab}	6.30±2.05^{ac}
OVERALL ACCEPTABILITY	0	6.49±1.68^{ab}	6.66±0.85^{ab}
	2.5	6.67±1.17^{ab}	6.56±0.82^{ab}
	5	6.40±1.64^{a}	6.16±1.24^{a}
	7.5	7.20±1.03^{a}	6.32±1.08^{a}

Means ± Standard deviations with different superscripts differ significantly ($P \leq 0.05$).
Based on a nine-point Hedonic scoring scale (9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor disliked, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely).

5.4. DISCUSSION

5.4.1. Effect of gamma irradiation on the microbial load of smoked guinea fowl meat during refrigeration storage period

The decrease microbial counts observed in the present study for the irradiated meat sample was expected, because gamma irradiation has been found to be an efficient method of reducing the number of bacteria in food products (Javanmard *et al.*, 2006). Studies have indicated that irradiation at doses of 3 kGy should yield 2 to 5 $\log_{10}$ reduction of pathogenic, non-spore forming bacteria (Lim *et al.*, 2007; Guinebretiere *et al.*, 2003). EFSA (2011), reported that, based on scientific evidence, the current recommendation for an overall average dose of 7 kGy and much lower doses would be sufficient to provide at least a 5-$\log_{10}$ reduction on the number of vegetative pathogens in frozen and chilled poultry products respectively. Also, irradiation in combination with other treatments suppresses the growth of surviving microorganisms during storage (Fan *et al.*, 2006; Caillet *et al.*, 2006; Quattara *et al.*, 2001). Since total viable counts and population of *Enterobacteriaceae* act as good hygienic quality indicators, their effects with irradiation were discussed.

5.4.1.1. Effect on total viable count

The number of viable counts detected (7.23 log10 cfu/g) in the control (0 kGy) smoked guinea fowl meat was higher than acceptable reference value of Ghana Standards Authority which prescribes values of $1.0*10^7$ cfu/g (GSA, 2008). These high microbial populations were reduced by the increasing gamma irradiation doses. The high prevalence of these viable counts could result from cross contamination

during handling and packaging of the meat, and not from inadequate processing condition.

Results of the current study indicated that gamma irradiation was effective in reducing the numbers of the total viable cells on smoked guinea fowl meat. Irradiation decreased TVC significantly ($p < 0.05$) with an overall observed value of 6.24 to 1.84 (log cfu/g). This result agreed with Haque *et al.* (2017), who reported a significant reduction of TVC in irradiated (2, 4 and 6 kGy) cooked beef. Similar results were reported by Ferawati *et al.* (2015) on fresh meat samples treated with 1, 2 and 3 kGy gamma irradiation dose. TVC of guinea fowl meat also decreased (4.68 to 2.68 log cfu/g) significantly ($p \leq 0.05$) with storage in the present study which did not agree with that of Haque *et al.* (2017), where storage period (up to 60 days) increased TVC of cooked beef. The decrease in TVC by gamma irradiation and storage in the present study could account for the bactericidal effects of smoke (Nollet and Toldra, 2008), which act as an antimicrobial agent in reducing the growth of microorganisms in the smoked guinea fowl meat during storage.

5.4.1.2. Effect on pathogens of Enterobacteriaceae

Gamma irradiation significantly reduced the pathogens of *Enterobacteriaceae* in the present study which agreed with Ouattara *et al.* (2001), who reported a significant reduction of *Enterobacteriaceae* on beef patties irradiated at 3 kGy. Since *E. coli* is a component of faecal microbiota, its enumeration indicated the occurrence of the two *Enterobacteriaceae* species (*S. marcescens* and *E. cloacae*) which are also pathogenic to man. A 2.5 kGy dose combined with refrigeration storage was able to reduce the

initial populations of *Enterobacteriaceae* species below the limit of detection at the 7 weeks of storage.

The results obtained compared favourably with values reported by Lescano *et al.* (1991), who did not detect *E. coli* in chicken meat irradiated at 2.5 kGy. Adu-Gyamfi *et al.* (2008), did not detect *E. coli* on poached chicken meal irradiated with 2.0 and 3.0 kGy during chill storage. Other studies also reported a 3-4 log cycle reduction of *E. coli* population in a 2 kGy irradiated chicken breast meat (Spoto *et al.*, 2000; Banati *et al.*, 1993). According to Banati *et al.* (1993), at low levels of contamination, a dose of 2 kGy is adequate to inactivate most of the non-spore forming bacteria in meals but, when contamination exceeds 10^6 $CFUg^{-1}$, higher doses of radiation are required to reduce the bacterial to acceptable counts, which could account for the reduction of the *Enterobacteriaceae* species in the present study.

5.4.2. Identification of Microbes in the smoked guinea fowl meat by the MALDI-TOF MS

5.4.2.1. Incidence of Staphylococcus aureus

Staphylococcus aureus was identified in the present study. Similar findings have been reported by Adzitey *et al.* (2015). However, contamination levels from the present study were comparatively higher than that of Adzitey *et al.* (2015) who reported contamination levels of 6.19 log cfu/cm^{-2} and 5.25 log cfu/cm^{-2} in fresh and smoked guinea fowl meat respectively. These authors reported *Staphylococcus* spp. as one of the most common identified bacteria found in smoked guinea fowl meat sampled from different retail shops. They attributed the high prevalence of this

pathogen to contamination at the slaughterhouse, either naturally or cross-contamination with infected carcasses. They also suggested that cross-contamination during transportation, packaging and handling of the meat products could have occurred.

S. aureus is one of the main pathogens detected in poultry meat, a major contaminant in food due to its high occurrence, and is a major cause of gastroenteritis in humans (Hennekinne *et al.*, 2012). The risk of disease caused by *S. aureus* is more related to unsuitable hygiene and storage throughout the food chain, as this bacterium has been reported to be found naturally in poultry meats and the environment (EFSA, 2012). On the other hand, the vegetative form of *S. aureus* has been reported to require temperatures above those used for refrigeration to grow to levels of concentration of public health relevance (EFSA, 2012). Thus, the occurrence of this pathogen in the present study was possible even during refrigeration storage, but with lower levels of concentration (Table 5.1).

S. aureus, being a mesophilic bacterium, has a relatively high heat resistance (Stewart, 2003). The presence of *S. aureus* in the treated guinea fowl meat in the current study could be attributed to their resistance to the smoking and irradiation processes. Besides, the packaging (HDPE pack) could also influence the presence of this pathogen. Though packaging serves as a physical barrier to microorganisms, packed products are also exposed to specific gas composition responsible for their growth (Rouger *et al.*, 2017). Optimization of the gas mixture in a package has been recommended, since the gas composition impacts the spoilage of the poultry meats (Rouger *et al.*, 2017).

5.4.2.2. Incidence of Enterobacteriaceae

It been reported that, several pathogens commonly associated with meat products belong to the family of *Enterobacteriaceae*, as meat products are occasionally spoiled by psychrotrophic members of this group of microorganisms during refrigerated storage (Quattara *et al.,* 2000). The presence of microorganisms in this family are well known indicator of hygienic problems, and has value in the assessment of the microbiological quality of foods (Alonso-Calleja *et al.,* 2002). *Serratia marcescens* and *Enterobacter cloacae* were the predominant *Enterobacteriaceae* detected in the present study. Similar report was made by Quattara *et al.* (2000), who detected *Serratia liquefaciens* as one of the *Enterobacteriaceae* present on bologna and pastrami meat products, which was substantially inhibited during storage. The low *Enterobacteriaceae* counts found in the present study agree with findings in various fermented sausages (Capita *et al.,* 2005; Drosinos *et al.,* 2005; Metaxopoulos *et al.,* 2001).

Enterobacteriaceae are known to be large group of related bacteria living in soil, water, and common inhabitant of both human and animal intestine (Yehia, 2013). They are also acquired through contaminated food or water, thus, being the major cause of diarrhoeal illness (Talaro and Talaro, 2002). Also, they are considered one of the dominate spoilage microorganisms in poultry meat (Chouliara *et al.,* 2008; Patsias *et al.,* 2008). Research has shown that, besides the well-known pathogens of the *Enterobacteriaceae* family, some members such as *Klebsiella* spp. and *Serratia* spp. among others, have been involved in human disease or caused opportunistic infections including bacteraemia, meningitis, urinary tract infections and wound infections (Darshan and Manonmani, 2015; Baylis *et al.,* 2011; Ashelford *et al.,* 2002).

Serratia marcescens (a primary pathogenic species of *Serratia*) is a rod-shaped, gram-negative nosocomial and opportunistic pathogen (Baylis *et al.*, 2011) classified in the tribe Klebsielleae and a member of the family *Enterobacteriaceae* (Khanna *et al.*, 2013). *Serratia* are identified to be widespread in the environment and thrive in diverse areas including water, soil, and the digestive tracts of various animals (Mlynarczyk *et al.*, 2007) as well as occasionally in the human intestinal tract (Baylis *et al.*, 2011). *S. marcescens* is known to be pathogenic to humans and a causative agent of contamination in hospital medical devices (Al-Ghanem, 2018). Although, these opportunistic pathogens are mostly associated with clinical settings (Baylis *et al.*, 2011), ingestion of contaminated foods (food spoilage) and direct contact are also modes of transmission (Grimont and Grimont, 1992). Thus, their presence in the guinea fowl meat may have occurred from work surfaces at the abattoir, transfer from soiled feathers onto the meat during evisceration or washing, and handling of the meat.

Enterobacter cloacae are known to occur in the intestinal tract of humans and animals (mostly poultry), water, soil, sewage, hospital environment, skin and meat (Yehia, 2013). This organism is often isolated from foods such as meats, poultry, dairy products and vegetables, however, beef and pork products are common reservoirs (Yehia, 2013). The presence of this organism in the guinea fowl meat may have resulted from cross-contamination from the intestinal tract of the fowls and handling during evisceration.

5.4.3. Effect of gamma irradiation on physicochemical properties of smoked guinea fowl meat during refrigeration storage period

5.4.3.1. Effect on pH

In the present study, increased pH was recorded among all the samples from week 5. The increase in pH was steady throughout the storage weeks at dose 5 kGy. The pH values for all the samples were within the neutral range with the exception of the samples irradiated at 5 kGy in the first week. This neutral range is numerically insignificant to affect quality characteristics of the processed meat product. During prolonged storage, meat suffers severe changes in terms of quality, one of which is increase in pH. Nester *et al.* (2007) proposed that a high pH favours microbial growth. That is, most bacteria will grow best at neutral pH (7), although they can tolerate ranges from 5 to 8 (Nester *et al.,* 2007). This implies that microorganisms are able to recover from the processing shock during storage under favourable pH condition. Also, increase in pH has been attributed to the process of proteolysis occurring in meat during storage (Surmei and Usturoi, 2012). The increase in pH values recorded for all the samples during storage from week 5 in the present study could also be due to the proteolytic process occurring during storage.

Badr and Mahmoud (2011) stated that a change in pH of irradiated meat products had little or no impact on their quality attributes. The pH trend observed in the current study agreed with that of Ham *et al.* (2017), who reported no marked change in the pH of processed cooked meat samples, irrespective of doses applied and the storage period. Similar results have been reported by Xavier *et al.* (2014) and Kanatt *et al.* (2015), who found that irradiation had no effect on the pH of beef trimmings (2 – 5 kGy) and chicken (2.5 – 10 kGy), respectively. Also, Nam *et al.* (2001 and 2002)

reported that gamma irradiation at 2.5 and 4.5 kGy did not affect the pH of aerobically packaged pork muscle and vacuum packaged pork types stored at 4 ^{o}C, respectively. In contrast, Dvořák *et al.* (2007) reported some increases in pH of fresh pheasant meat upon irradiation (2.5 and 5 kGy) at a temperature of 6 ^{o}C. The disparity from the study could result from the meat type, processing condition and storage.

5.4.3.2. Effect on titratable acidity

The titratable acidity (TA) is a better predictor of acid impact on flavour. The changes in TA of all the samples had no specific pattern since there was no dose dependent effect of gamma irradiation and storage weeks. Immediately after irradiation, the total acidity of the meat samples was reduced significantly for dose 2.5 – 5.0 kGy, but increased at the highest dose of 7.5 kGy. Generally the TA increased significantly with storage. Similar report was seen by Adeyinka *et al.* (2011) who reported an increased total titratable acidity of dried meat samples under both refrigerated and non-refrigerated storage conditions for a period of 5 weeks. Park *et al.* (2013) also, observed a linear increase of TA with storage time in chicken breast meat during different storage periods (5, 10 and 15 ^{o}C). The acetic acid production from bacterial growth and metabolism could account for the rise of TA with storage in the present study. Similar result was reported by Park *et al.* (2013), who explained colour changes in growth media to result from lactic acid production from bacterial growth and metabolism in a time-temperature indicator (TTI) response of chicken breast meat.

5.4.3.3. Effect on acid value (AV)

Acid value (AV) represents the free fatty acid content of food due to enzymatic activity (which can be from the tissue from which the fat is extracted or microbial contamination), which is usually an indicative of spoilage (Al-Bachir, 2013). The acid value of the control samples decreased gradually with storage but increased significantly at the 7th week (8.60 – 9.35 mg/g). This increase could possibly be attributed to the recovering and growth of microorganisms from refrigerated temperature shock during storage. Jay (1992) stated that, formation of volatile compounds during storage causes an increase in the volatile basic nitrogen value, which is strongly linked to the growth of microorganisms.

Generally, gamma irradiation significantly decreased the AV of the smoked guinea fowl meat but increased as storage progressed. However, during storage, AV for the irradiated smoked guinea fowl meat increased. Ham *et al.* (2017) reported increase of Thiobarbituric acid reactive substances (TBARS) values of cooked beef patties when processed with gamma irradiation (2, 4 and 6 kGy). Hydroxyl radicals generated by ionizing radiation are well documented as a factor in accelerating lipid oxidation (Devasagayam, *et al.*, 2004). Studies have also indicated that acceleration of lipid oxidation in irradiated meat products depends on irradiation dose levels (Song *et al.*, 2009). In the present study, the increase in AV was profound in the 2.5 kGy sample, where a steady increase of acid value with storage was observed. In this regard, it can be deduced from the present study that the extent of lipid oxidation is affected by irradiation dose and storage. Similarly, the AV for 5 and 7.5 kGy samples increased steadily from the 5th week. The results from the present study indicates that AV

increases in all the irradiated meat samples during storage. The increases in the AV could be due to slight random hydrolysis of triglycerol molecules to free fatty acids and diacylglycerols, as reported by Al-Bachir (2004). The oxidation of oils and fats is known to be one of the main sources of deterioration of organoleptic and nutritional characteristics of food stuff (Ghosh *et al.*, 2014).

5.4.4. The relationship between pH, total acidity and acid value

There was a positive correlation at 5% confidence level between acid value and total acidity of irradiated smoked guinea fowl meat under refrigeration storage. The strong linear positive correlation (0.934) between acid value and total titratable acidity is an indication that, acidity in meat increases with storage although, not statistically significant ($p = 0.06$). The increase in these values could mainly be attributed to rancidity occurring in the meat during storage (Haque *et al.*, 2017; Morales *et al.*, 2009; Al-Bachir and Zeinou, 2009; Chen *et al.*, 2007).

On the other hand, a strong negative correlation between pH and acid value (-0.81) and pH and total acidity (-0.85) implies that pH negatively influenced the acid value (AV) and titratable acidity (TA) of meat during storage. That is, as AV and TA increases, pH decreases. Similar reports were made by Morales *et al.* (2009) where increased fat values in irradiated meat samples caused decreased pH of raw goat meat. Haque *et al.* (2017) also reported a decreased pH throughout storage period due to increased free fatty acids. These authors reported these changes to result from rancidity occurring in the meat as storage progressed. On the contrary, a highly insignificant positive correlation (0.128) between the pH and the doses contradict that

of Aftab *et al.* (2015). Aftab *et al.* (2015) found that the pH of raw meat decreased with higher irradiation as well as storage period. The type of meat and processing conditions could account for the disparity in the present study.

5.4.5. Effect of irradiation on the sensory (organoleptic) properties of smoked guinea fowl meat stored at refrigeration conditions

Balamatsia *et al.* (2006) stated that the original quality of poultry meat can be evaluated by organoleptic (sensory) properties besides physical and chemical analysis. Recent studies have also shown irradiation to impart detrimental organoleptic attributes to high-fat products (Norhana *et al.*, 2010).

5.4.5.1. Effect on colour

In general, irradiation effect on the colour of the meat samples was not significant. The colour of both irradiated and non-irradiated meat samples were judged equally. However, the colour for 7.5 kGy sample at month 1 was moderately liked but liked slightly at the end of the storage period. Similar observation was recorded in beef (Haque *et al.*, 2017). The total colour change of the irradiated meat samples was also not significantly different ($p > 0.05$) from non-irradiated ones. Nam and Ahn (2002) found that colour values of irradiated and non-irradiated meat were similar, which agreed with the present study. These authors, as reported by Brewer (2004) attributed the outcome to myoglobin molecule in muscle meat, which is altered with the chemical environment and the energy input. On the contrary, irradiation did not increase the intensity of the colour of the smoked guinea fowl meat, which is in disagreement with reports by Haque *et al.* (2017) and Miller *et al.* (1995).

5.4.5.2. Effect on tenderness

The tenderness/texture for all the samples were equally liked during the storage period, although, samples irradiated at 7.5 kGy were liked moderately during the initial month (month 0). There were no significant differences ($p > 0.05$) between the tenderness of irradiated and non-irradiated samples. Hashim *et al.* (1995) reported that cooked irradiated chicken samples were tenderer than non-irradiated samples. Haque *et al.* (2017), also reported slight increase in tenderness with irradiation of beef, with significant decrease during storage. The results observed for tenderness of irradiated smoked guinea fowl meat in the present study varied from those reported by Hashim *et al.* (1995) and Haque *et al.* (2017) for cooked irradiated chicken and irradiated beef, respectively. These disparities could be attributed to the type of poultry meat, part of the meat used, and type of processing method.

5.4.5.3. Effect on aroma

The aroma for all the samples was equally liked, however, samples irradiated at 7.5 kGy had the highest score value of 7.05 and the least value of 5.85 recorded for 5 kGy at month 1. Similar observations have been reported by Haque *et al.* (2017) and Modi *et al.* (2008). Minimal changes in flavour of cooked beef and goat meat due to gamma irradiation (2, 4 and 6 kGy) have been reported by Haque *et al.* (2017) and Modi *et al.* (2008), respectively. A review paper by Jung (2007), on effect of irradiation on meat colour, suggested that irradiation produces some radiolytic products that are responsible for off-odour in meat. Production of off-odours and off-flavours in beef due to radiolytic effect have been related to radiation dose, dose rate, temperature and packaging condition (Jung, 2007).

5.4.5.4. Effect on taste

The taste for all the samples (0, 2.5, 5.0 and 7.5 kGy) at month 0 (week 4) was equally liked however; sample irradiated at 7.5 kGy was more liked. However, significant differences were observed for taste among the samples after the two month storage period. Non-irradiated meat samples were judged to be tastier than irradiated samples, with 5 kGy samples having the least preference. This implies that, though irradiation did not affect the taste of the meat samples, storage may have imparted an unfavourable taste to the irradiated meat samples. Irradiated meat samples seemed to be less preferred at the end of storage period with 5 kGy sample having the least likeness. The results from the present study were in disparity with those reported by Torgby-Tetteh (2010), who reported favourable taste to cooked irradiated chicken meat at 2.0 and 6.0 kGy, comparable to 0 and 4.0 kGy with no consistent effect of storage on the meat. The above differences could be attributed to type of poultry meat used and the processing condition as well as the irradiation dose used.

5.4.5.5. Overall Acceptability

Refrigeration storage condition had no significant effect on overall acceptability of both irradiated and non-irradiated smoked guinea fowl meat. Sample treated with 7.5 kGy was liked moderately at the initial month (month 0) compared with other samples, whereas control sample (0 kGy) was rated higher than other samples at the end of storage period. Haque *et al.* (2017) reported no significant effect on the overall acceptability of irradiated and non-irradiated beef, but the overall acceptability decreased significantly with storage time, which is contrary to results obtained from the current study. Likewise, Johnson *et al.* (2004) reported similar observation to

Haque *et al.* (2017). However, earlier studies had reported on irradiated chicken at doses 2.5 to 10 kGy to have no effect on the acceptability of either raw or cooked chicken for appearance, odour, texture, or taste (Abu-Tarboush *et al.*, 1997).

5.5. CONCLUSION

This study aimed to assess the quality of smoked guinea fowl meat processed with gamma irradiation at doses of 0, 2.5, 5 and 7.5 kGy. The study showed a significant additive interaction effect of gamma irradiation with smoking in reducing the growth of bacteria in smoked guinea fowl meat. The effect was characterized by lower growth rates with a resulting significant shelf life extension in irradiated meat samples. Gamma irradiation reduced drastically the population of microorganisms and increased the shelf life of refrigerated smoked guinea fowl meat during the two month (8 weeks) storage period. The pH values of the meat samples were not affected by gamma irradiation. Gamma irradiation significantly affected the titratable acidity and acid value in a non-dose dependent manner. On the basis of organoleptic properties, irradiation enhanced the sensory qualities of the smoked guinea fowl meat; however the taste of the irradiated meat samples was influenced during the storage period. The overall acceptability scores for the meat sample were liked moderately during the storage period, but were not significantly affected. Generally, gamma irradiation showed a potential tool to be used to enhance the shelf life of smoked guinea fowl meat, and it is recommended that a dose of 5 kGy as a safety dose of radiation with adequate refrigeration should be considered for the treatment of smoked guinea fowl meat aiming at it conservation.

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CHAPTER SIX

6. GENERAL CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The study showed that the shelf life and quality of smoked guinea fowl meat have been enhanced by gamma irradiation. In assessing the gamma irradiation (0, 2.5, 5.0 and 7.5 kGy) effect on the quality and shelf life of the smoked guinea fowl meat, the nutritional quality, microbiological, physicochemical, sensory and smoke quality (PAHs concentrations) of the meat were evaluated.

The macronutrients contents (proximate composition) of the smoked guinea fowl meat were affected by the irradiation doses applied. The mineral compositions of the smoked guinea fowl meat samples were however not significantly affected by gamma irradiation doses applied.

Sixteen PAHs as denoted as U.S. EPA 16 PAHs were detected in the smoked guinea fowl meat. The total PAHs consisted of 10 low molecular weight (LMW) and 6 high molecular weight (HMW) PAHs with LMW PAHs dominating although their concentrations were less than that of HMW PAHs. Anthracene and Benzo[a]pyrene were the most occurring LMW and HMW PAHs, respectively. Concentration of B[a]P as a marker for carcinogenic PAH in smoked foods was higher than the acceptable limit however, the concentration decreased considerably with increasing gamma irradiation dose. The PAH4 as a suitable indicator of PAHs in food was however below the permissible level in all the treated meat samples. Concentrations of all PAHs

and their carcinogenic human health risk assessment indicators in the meat samples reduced exponentially with the increasing doses of gamma irradiation.

Food-borne microorganisms of public health importance were isolated in the smoked guinea fowl meat. Bacterial isolates identified were *Staphylococcus aureus, Serratia marcescens* and *Enterobacter cloacae*. Gamma irradiation significantly reduced microbial populations in dose-dependent manner. The microbial loads of the smoked guinea fowl meat (irradiated and non-irradiated) were also reduced at the refrigeration storage period.

The physicochemical properties of smoked guinea fowl meat were slightly affected by irradiation during refrigeration storage period. Gamma irradiation did not affect the pH of the smoked guinea fowl meat; however the pH of the meat samples were within the neutral range which was numerically insignificant to affect quality characteristics of the processed meat. Titratable acidity (TA) and acid value (AV) decreased significantly with gamma irradiation, but increased with storage. A significant strong positive correlation existed between the acid value and titratable acidity of the meat samples over the storage period.

Gamma irradiation enhanced the sensory qualities of the smoked guinea fowl meat, however the taste of the irradiated (5 and 7.5 kGy) meat samples were affected during the storage period. The overall acceptability scores for the meat samples were liked moderately during the storage period, and were not significantly different.

6.2. RECOMMENDATIONS

Based on the findings of this study and considering future research, the following recommendations are made:

1. Irradiation dose of 5 kGy is recommended for use to considerably reduce most of the carcinogenic PAHs and foodborne pathogens, with minimal effect on nutritional, physicochemical and sensory qualities of smoked guinea fowl meat during storage.
2. Further investigation on gamma irradiation effect on the molecular structure of various PAHs compounds should be undertaken.
3. Thiobarbituric acid reactive substances (TBARs) and peroxide value (PV), as lipid oxidation indices should be considered during physicochemical analysis that would best elucidate biochemical changes occurring in meat during storage.
4. Further studies should be carried out on the amino acids and fatty acids profiles and other volatiles in the smoked guinea fowl meat, which are necessary components in food diet and health.
5. Irradiation in combination with vacuum packaging should be investigated for storing smoked guinea fowl meat at ambient condition for a shelf stable meat product.

CHAPTER SEVEN

7. APPENDICES

Appendix 1

a. One-way ANOVA for proximate composition in smoked guinea fowl meat irradiated with different doses of gamma irradiation

ANOVA Table for %Moisture by Dose (kGy)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	144.025	3	48.0084	338.42	0.0000
Within groups	0.56745	4	0.141863		
Total (Corr.)	144.593	7			

ANOVA Table for %Ash by Dose (kGy)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.07474	3	0.358246	52.01	0.0012
Within groups	0.02755	4	0.0068875		
Total (Corr.)	1.10229	7			

ANOVA Table for %Fat by Dose (kGy)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	385.6	3	128.533	1191.36	0.0000
Within groups	0.43155	4	0.107888		
Total (Corr.)	386.032	7			

ANOVA Table for %Protein by Dose (kGy)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	179.862	3	59.9539	582.15	0.0000
Within groups	0.41195	4	0.102988		
Total (Corr.)	180.274	7			

ANOVA Table for Carbohydrate by Dose (kGy)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1051.07	3	350.357	1272.69	0.0000
Within groups	1.10115	4	0.275287		
Total (Corr.)	1052.17	7			

ANOVA Table for Energy by Dose (kGy)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	10113.5	3	3371.15	879.43	0.0000
Within groups	15.3333	4	3.83333		
Total (Corr.)	10128.8	7			

Appendix 1b

(a) Quantitative analysis of spectrum deconvolution and computation of elemental concentration in smoked guinea fowl meat.

Fit Parameters (control) :

FIT parameters	
Region of Fit	1 – 1023
Number of iterations	5
Chi square	156.0546
Last Chi square difference	0.1608 %
Calibration parameters	
Zero	-9.38809E-02 +/- 5.76362E-04
Gain	2.66341E-02 +/- 3.21173E-06
Noise	1.40300E-01 +/- 1.39169E-03
Fano	1.93763E-01 +/- 1.04364E-02
Sum	0.00000E+00 +/- 0.00000E+00
Continuum parameters	
Type	Exp. Polynomial
A0	6.05171E+03 +/- 6.39910E+00
A1	-4.36432E-02 +/- 3.50647E-04
A2	-7.18206E-03 +/- 9.84054E-05
A3	5.88288E-04 +/- 1.26531E-05
A4	-6.04120E-05 +/- 1.89870E-06
A5	3.47284E-06 +/- 1.04500E-07
A6	-2.77130E-07 +/- 1.01429E-08

Fit Parameters (irradiated sample):

FIT parameters	
Region of Fit	0 – 1023
Number of iterations	5
Chi square	175.9379
Last Chi square difference	0.1024 %
Calibration parameters	
Zero	-9.30505E-02 +/- 6.15295E-04
Gain	2.66321E-02 +/- 3.32829E-06
Noise	1.49993E-01 +/- 8.41725E-03
Fano	2.11516E-01 +/- 7.46525E-03
Sum	0.00000E+00 +/- 0.00000E+00

Continuum parameters	
Type	Exp. Polynomial
A0	6.02101E+03 +/- 6.28543E+00
A1	-9.96423E-03 +/- 3.53986E-04
A2	-4.18118E-03 +/- 9.45703E-05
A3	4.81665E-04 +/- 1.26346E-05
A4	-1.06889E-04 +/- 1.82043E-06
A5	3.12493E-06 +/- 9.91987E-08
A6	-4.47626E-08 +/- 9.44462E-09

(b) ANOVA tables for elemental analysis of irradiated and non-irradiated smoked guinea fowl meat samples.

ANOVA Table for 0 kGy by Element

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	21214.7	18	1178.59	35160.34	0.0000
Within groups	0.636891	19	0.0335206		
Total (Corr.)	21215.3	37			

ANOVA Table for 2.5 kGy by Element

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	27441.8	18	1524.54	48785.34	0.0000
Within groups	0.59375	19	0.03125		
Total (Corr.)	27442.4	37			

ANOVA Table for 5.0 kGy by Element

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	27512.9	18	1528.5	53178.06	0.0000
Within groups	0.546117	19	0.028743		
Total (Corr.)	27513.5	37			

ANOVA Table for 7.5 kGy by Element

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	27549.1	18	1530.51	27978.42	0.0000
Within groups	1.03936	19	0.0547031		
Total (Corr.)	27550.2	37			

Appendix 2

a. **One-Way ANOVA summary showing significant differences at 95% confidence level in means of PAHs in smoked guinea fowl meat treated with different doses of gamma radiation.**

ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	149.183	3	49.7278	4.87	0.0030
Within groups	1429.85	140	10.2132		
Total (Corr.)	1579.03	143			

Appendix 3

a. Factorial ANOVA showing interaction between storage period (0, 2, 5, and 7 weeks) and doses (0, 2.5, 5.0, 7.5 kGy) of samples irradiated for Total Viable Counts (TVC).

	Univariate Tests of Significance for TVC (Spreadshee Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	567.1395	1	567.1395	72863.11	0.000000
DOSE	138.5382	3	46.1794	5932.89	0.000000
STORAGE TIME	27.2716	3	9.0905	1167.90	0.000000
DOSE*STORAGE TIME	2.3372	9	0.2597	33.36	0.000000
Error	0.2491	32	0.0078		

	Descriptive Statistics (Spreadsheet1)							
Effect	Level of Factor	Level of Factor	N	TVC Mean	TVC Std.Dev.	TVC Std.Err	TVC -95.00%	TVC +95.00%
Total			48	3.437355	1.892854	0.273210	2.887727	3.986982
DOSE	1		12	6.243963	0.623170	0.179894	5.848020	6.639906
DOSE	2.5		12	3.275426	1.101704	0.318034	2.575437	3.975415
DOSE	5		12	2.383730	0.906936	0.261810	1.807490	2.959969
DOSE	7.5		12	1.846299	0.538265	0.155384	1.504302	2.188297
STORAGE TIME	0		12	4.686703	1.757514	0.507350	3.570032	5.803374
STORAGE TIME	2		12	3.303333	1.785001	0.515285	2.169198	4.437469
STORAGE TIME	5		12	3.069195	1.792247	0.517377	1.930456	4.207935
STORAGE TIME	7		12	2.690187	1.828187	0.527752	1.528613	3.851761
DOSE*STORAGE TIME	1	0	3	7.227866	0.005140	0.002967	7.215098	7.240635
DOSE*STORAGE TIME	1	2	3	6.149738	0.136985	0.079088	5.809448	6.490027
DOSE*STORAGE TIME	1	5	3	5.928198	0.061965	0.035776	5.774268	6.082128
DOSE*STORAGE TIME	1	7	3	5.670049	0.064783	0.037402	5.509120	5.830978
DOSE*STORAGE TIME	2.5	0	3	5.002852	0.006606	0.003814	4.986442	5.019263
DOSE*STORAGE TIME	2.5	2	3	3.077694	0.060048	0.034669	2.928526	3.226861
DOSE*STORAGE TIME	2.5	5	3	2.855056	0.018402	0.010625	2.809342	2.900770
DOSE*STORAGE TIME	2.5	7	3	2.166104	0.161342	0.093151	1.765309	2.566898
DOSE*STORAGE TIME	5	0	3	3.842986	0.007164	0.004136	3.825188	3.860783
DOSE*STORAGE TIME	5	2	3	2.166104	0.161342	0.093151	1.765309	2.566898
DOSE*STORAGE TIME	5	5	3	1.900810	0.054608	0.031528	1.765157	2.036464
DOSE*STORAGE TIME	5	7	3	1.625020	0.128084	0.073950	1.306841	1.943200
DOSE*STORAGE TIME	7.5	0	3	2.673108	0.052419	0.030264	2.542892	2.803324
DOSE*STORAGE TIME	7.5	2	3	1.819797	0.072133	0.041646	1.640608	1.998987
DOSE*STORAGE TIME	7.5	5	3	1.592717	0.111219	0.064212	1.316434	1.869001
DOSE*STORAGE TIME	7.5	7	3	1.299575	0.043593	0.025169	1.191283	1.407867

b. Factorial ANOVA showing the interaction between storage (0, 2, 5, and 7 weeks) and doses (0, 2.5, 5.0, 7.5 kGy) of samples irradiated for *E.coli*.

	Univariate Tests of Significance for E.COLI (Spreadshee Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	32.55055	1	32.55055	891.2087	0.000000
DOSE	38.80899	3	12.93633	354.1866	0.000000
STORAGE TIME	0.82059	3	0.27353	7.4890	0.000622
DOSE*STORAGE TIME	1.80168	9	0.20019	5.4809	0.000146
Error	1.16877	32	0.03652		

	Descriptive Statistics (Spreadsheet1)							
Effect	Level of Factor	Level of Factor	N	E.COLI Mean	E.COLI Std.Dev.	E.COLI Std.Err	E.COLI -95.00%	E.COLI +95.00%
Total			48	0.823490	0.952042	0.137415	0.54705	1.099935
DOSE	1		12	2.157635	0.201209	0.058084	2.02979	2.285477
DOSE	2.5		12	1.136326	0.551502	0.159205	0.78592	1.486734
DOSE	5		12	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE	7.5		12	0.000000	0.000000	0.000000	0.00000	0.000000
STORAGE TIME	0		12	0.924685	1.016312	0.293384	0.27895	1.570418
STORAGE TIME	2		12	0.915675	1.012259	0.292214	0.27252	1.558834
STORAGE TIME	5		12	0.851193	0.936308	0.270289	0.25629	1.446095
STORAGE TIME	7		12	0.602409	0.929461	0.268312	0.01186	1.192961
DOSE*STORAGE TIME	1	0	3	2.206364	0.358533	0.206999	1.31572	3.097010
DOSE*STORAGE TIME	1	2	3	2.264992	0.201185	0.116154	1.76522	2.764764
DOSE*STORAGE TIME	1	5	3	2.096678	0.089306	0.051561	1.87483	2.318527
DOSE*STORAGE TIME	1	7	3	2.062507	0.075317	0.043485	1.87541	2.249606
DOSE*STORAGE TIME	2.5	0	3	1.492374	0.199408	0.115128	0.99702	1.987731
DOSE*STORAGE TIME	2.5	2	3	1.397708	0.017382	0.010036	1.35453	1.440888
DOSE*STORAGE TIME	2.5	5	3	1.308093	0.012234	0.007063	1.27770	1.338483
DOSE*STORAGE TIME	2.5	7	3	0.347131	0.601248	0.347131	-1.14645	1.840715
DOSE*STORAGE TIME	5	0	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	5	2	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	5	5	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	5	7	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	0	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	2	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	5	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	7	3	0.000000	0.000000	0.000000	0.00000	0.000000

c. Factorial ANOVA showing the interaction between storage (0, 2, 5, and 7 weeks) and doses (0, 2.5, 5.0, and 7.5 kGy) of samples irradiated for *Staph aureus*.

	Univariate Tests of Significance for STAPH (Spreadsheet Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	84.08901	1	84.08901	2257.159	0.000000
DOSE	81.73908	3	27.24636	731.360	0.000000
STORAGE TIME	8.90162	3	2.96721	79.647	0.000000
DOSE*STORAGE TIME	5.15104	9	0.57234	15.363	0.000000
Error	1.19214	32	0.03725		

	Descriptive Statistics (Spreadsheet1)							
Effect	Level of Factor	Level of Factor	N	STAPH Mean	STAPH Std.Dev.	STAPH Std.Err	STAPH -95.00%	STAPH +95.00%
Total			48	1.323576	1.436484	0.207339	0.90646	1.740688
DOSE	1		12	3.248209	0.693983	0.200336	2.80727	3.689145
DOSE	2.5		12	1.789498	0.829706	0.239516	1.26233	2.316668
DOSE	5		12	0.256598	0.464613	0.134122	-0.03860	0.551800
DOSE	7.5		12	0.000000	0.000000	0.000000	0.00000	0.000000
STORAGE TIME	0		12	1.941616	1.598437	0.461429	0.92602	2.957214
STORAGE TIME	2		12	1.484959	1.680300	0.485061	0.41735	2.552571
STORAGE TIME	5		12	1.056409	1.208184	0.348773	0.28877	1.824052
STORAGE TIME	7		12	0.811321	1.081371	0.312165	0.12425	1.498391
DOSE*STORAGE TIME	1	0	3	3.952464	0.014725	0.008501	3.91589	3.989042
DOSE*STORAGE TIME	1	2	3	3.843159	0.050001	0.028868	3.71895	3.967369
DOSE*STORAGE TIME	1	5	3	2.777636	0.025830	0.014913	2.71347	2.841800
DOSE*STORAGE TIME	1	7	3	2.419578	0.092033	0.053135	2.19095	2.648201
DOSE*STORAGE TIME	2.5	0	3	2.787607	0.010777	0.006222	2.76084	2.814378
DOSE*STORAGE TIME	2.5	2	3	2.096678	0.089306	0.051561	1.87483	2.318527
DOSE*STORAGE TIME	2.5	5	3	1.448000	0.076017	0.043888	1.25916	1.636835
DOSE*STORAGE TIME	2.5	7	3	0.825707	0.753827	0.435222	-1.04690	2.698317
DOSE*STORAGE TIME	5	0	3	1.026394	0.045715	0.026394	0.91283	1.139957
DOSE*STORAGE TIME	5	2	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	5	5	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	5	7	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	0	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	2	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	5	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	7	3	0.000000	0.000000	0.000000	0.00000	0.000000

d. Factorial ANOVA showing the interaction between storage (0, 2, 5, and 7 weeks) and doses (0, 2.5, 5.0, and 7.5 kGy) of samples irradiated for *Bacillus cereus*.

	Univariate Tests of Significance for BC (Spreadshee Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	65.94139	1	65.94139	1001.101	0.000000
DOSE	57.23568	3	19.07856	289.645	0.000000
STORAGE TIME	10.90687	3	3.63562	55.195	0.000000
DOSE*STORAGE TIME	4.52934	9	0.50326	7.640	0.000007
Error	2.10780	32	0.06587		

	Descriptive Statistics (Spreadsheet1)							
Effect	Level of Factor	Level of Factor	N	BC Mean	BC Std.Dev.	BC Std.Err	BC -95.00%	BC +95.00%
Total			48	1.172083	1.261371	0.182063	0.80582	1.538347
DOSE	1		12	2.735348	0.813457	0.234825	2.21850	3.252194
DOSE	2.5		12	1.638138	0.777126	0.224337	1.14438	2.131900
DOSE	5		12	0.314846	0.573824	0.165649	-0.04974	0.679436
DOSE	7.5		12	0.000000	0.000000	0.000000	0.00000	0.000000
STORAGE TIME	0		12	1.964151	1.569856	0.453178	0.96671	2.961590
STORAGE TIME	2		12	1.117670	1.238028	0.357388	0.33106	1.904275
STORAGE TIME	5		12	0.860288	0.955255	0.275758	0.25335	1.467228
STORAGE TIME	7		12	0.746223	0.947073	0.273396	0.14448	1.347964
DOSE*STORAGE TIME	1	0	3	3.983460	0.020293	0.011716	3.93305	4.033871
DOSE*STORAGE TIME	1	2	3	2.787607	0.010777	0.006222	2.76084	2.814378
DOSE*STORAGE TIME	1	5	3	2.123072	0.127881	0.073832	1.80540	2.440746
DOSE*STORAGE TIME	1	7	3	2.047254	0.096077	0.055470	1.80859	2.285923
DOSE*STORAGE TIME	2.5	0	3	2.613761	0.496055	0.286397	1.38149	3.846030
DOSE*STORAGE TIME	2.5	2	3	1.683073	0.140318	0.081013	1.33450	2.031642
DOSE*STORAGE TIME	2.5	5	3	1.318081	0.275466	0.159040	0.63379	2.002377
DOSE*STORAGE TIME	2.5	7	3	0.937638	0.812063	0.468845	-1.07964	2.954914
DOSE*STORAGE TIME	5	0	3	1.259384	0.163409	0.094344	0.85345	1.665315
DOSE*STORAGE TIME	5	2	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	5	5	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	5	7	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	0	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	2	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	5	3	0.000000	0.000000	0.000000	0.00000	0.000000
DOSE*STORAGE TIME	7.5	7	3	0.000000	0.000000	0.000000	0.00000	0.000000

Appendix 4

a. Shelf life study of pH in smoked guinea fowl meat during a two month storage period.

Effect	Univariate Tests of Significance for pH (Spreadsheet3 Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	2428.777	1	2428.777	15754226	0.000000
DOSE	0.051	3	0.017	109	0.000000
STORAGE TIME	0.089	3	0.030	192	0.000000
DOSE*STORAGE TIME	0.195	9	0.022	140	0.000000
Error	0.005	32	0.000		

Effect	Descriptive Statistics (Spreadsheet35)							
	Level of Factor	Level of Factor	N	pH Mean	pH Std.Dev.	pH Std.Err	pH -95.00%	pH +95.00%
Total			48	7.113333	0.084986	0.012267	7.088656	7.138011
DOSE	0		12	7.105000	0.072174	0.020835	7.059143	7.150857
DOSE	2.5		12	7.135833	0.113415	0.032740	7.063773	7.207894
DOSE	5		12	7.064167	0.063883	0.018441	7.023577	7.104756
DOSE	7.5		12	7.148333	0.064079	0.018498	7.107620	7.189047
STORAGE TIME	0		12	7.113333	0.094708	0.027340	7.053158	7.173508
STORAGE TIME	2		12	7.062500	0.054793	0.015817	7.027686	7.097314
STORAGE TIME	5		12	7.096667	0.063437	0.018313	7.056361	7.136973
STORAGE TIME	7		12	7.180833	0.082292	0.023756	7.128548	7.233119
DOSE*STORAGE TIME	0	0	3	7.190000	0.000000	0.000000	7.190000	7.190000
DOSE*STORAGE TIME	0	2	3	7.050000	0.010000	0.005774	7.025159	7.074841
DOSE*STORAGE TIME	0	5	3	7.026667	0.020817	0.012019	6.974955	7.078378
DOSE*STORAGE TIME	0	7	3	7.153333	0.005774	0.003333	7.138991	7.167676
DOSE*STORAGE TIME	2.5	0	3	7.070000	0.010000	0.005774	7.045159	7.094841
DOSE*STORAGE TIME	2.5	2	3	7.013333	0.005774	0.003333	6.998991	7.027676
DOSE*STORAGE TIME	2.5	5	3	7.160000	0.000000	0.000000	7.160000	7.160000
DOSE*STORAGE TIME	2.5	7	3	7.300000	0.017321	0.010000	7.256973	7.343027
DOSE*STORAGE TIME	5	0	3	6.986667	0.020817	0.012019	6.934955	7.038378
DOSE*STORAGE TIME	5	2	3	7.036667	0.005774	0.003333	7.022324	7.051009
DOSE*STORAGE TIME	5	5	3	7.150000	0.010000	0.005774	7.125159	7.174841
DOSE*STORAGE TIME	5	7	3	7.083333	0.011547	0.006667	7.054649	7.112018
DOSE*STORAGE TIME	7.5	0	3	7.206667	0.005774	0.003333	7.192324	7.221009
DOSE*STORAGE TIME	7.5	2	3	7.150000	0.000000	0.000000	7.150000	7.150000
DOSE*STORAGE TIME	7.5	5	3	7.050000	0.026458	0.015275	6.984276	7.115724
DOSE*STORAGE TIME	7.5	7	3	7.186667	0.005774	0.003333	7.172324	7.201009

b. Shelf life study on TTA in smoked guinea fowl meat during a two month storage period.

Effect	Univariate Tests of Significance for TTA (Spreadshee Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	10.20285	1	10.20285	19280.98	0.000000
Dose	0.02571	3	0.00857	16.19	0.000001
Storage	0.18792	3	0.06264	118.38	0.000000
Dose*Storage	0.12489	9	0.01388	26.22	0.000000
Error	0.01693	32	0.00053		

Effect	Descriptive Statistics (Spreadsheet2) Level of Factor	Level of Factor	N	TTA Mean	TTA Std.Dev.	TTA Std.Err	TTA -95.00%	TTA +95.00%
Total			48	0.461042	0.086964	0.012552	0.435790	0.486293
Dose	0.0		12	0.499167	0.068285	0.019712	0.455780	0.542553
Dose	2.5		12	0.446667	0.070108	0.020238	0.402122	0.491211
Dose	5.0		12	0.459167	0.120714	0.034847	0.382468	0.535865
Dose	7.5		12	0.439167	0.076332	0.022035	0.390668	0.487665
Storage	0		12	0.435833	0.075493	0.021793	0.387867	0.483800
Storage	2		12	0.380000	0.046122	0.013314	0.350695	0.409305
Storage	5		12	0.476667	0.069194	0.019975	0.432703	0.520631
Storage	7		12	0.551667	0.051139	0.014762	0.519175	0.584159
Dose*Storage	0.0	0	3	0.473333	0.005774	0.003333	0.458991	0.487676
Dose*Storage	0.0	2	3	0.446667	0.020817	0.012019	0.394955	0.498378
Dose*Storage	0.0	5	3	0.466667	0.005774	0.003333	0.452324	0.481009
Dose*Storage	0.0	7	3	0.610000	0.000000	0.000000	0.610000	0.610000
Dose*Storage	2.5	0	3	0.413333	0.005774	0.003333	0.398991	0.427676
Dose*Storage	2.5	2	3	0.356667	0.011547	0.006667	0.327982	0.385351
Dose*Storage	2.5	5	3	0.486667	0.011547	0.006667	0.457982	0.515351
Dose*Storage	2.5	7	3	0.530000	0.010000	0.005774	0.505159	0.554841
Dose*Storage	5.0	0	3	0.333333	0.015275	0.008819	0.295388	0.371279
Dose*Storage	5.0	2	3	0.363333	0.045092	0.026034	0.251317	0.475349
Dose*Storage	5.0	5	3	0.566667	0.032146	0.018559	0.486813	0.646521
Dose*Storage	5.0	7	3	0.573333	0.049329	0.028480	0.450794	0.695873
Dose*Storage	7.5	0	3	0.523333	0.030551	0.017638	0.447442	0.599225
Dose*Storage	7.5	2	3	0.353333	0.011547	0.006667	0.324649	0.382018
Dose*Storage	7.5	5	3	0.386667	0.023094	0.013333	0.329298	0.444035
Dose*Storage	7.5	7	3	0.493333	0.015275	0.008819	0.455388	0.531279

c. Shelf life study on AV in smoked guinea fowl meat during a two month storage period.

Effect	Univariate Tests of Significance for AV (Spreadsheet3 Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	2952.090	1	2952.090	14070.03	0.000000
DOSE	27.715	3	9.238	44.03	0.000000
STORAGE TIME	28.030	3	9.343	44.53	0.000000
DOSE*STORAGE TIME	19.677	9	2.186	10.42	0.000000
Error	6.714	32	0.210		

Effect	Descriptive Statistics (Spreadsheet35)							
	Level of Factor	Level of Factor	N	AV Mean	AV Std.Dev.	AV Std.Err	AV -95.00%	AV +95.00%
Total			48	7.842313	1.321956	0.190808	7.458457	8.22617
DOSE	0		12	8.368250	0.970453	0.280146	7.751654	8.98485
DOSE	2.5		12	7.760500	1.091845	0.315188	7.066775	8.45423
DOSE	5		12	8.602000	0.966761	0.279080	7.987750	9.21625
DOSE	7.5		12	6.638500	1.370689	0.395684	5.767606	7.50939
STORAGE TIME	0		12	7.199500	1.391406	0.401664	6.315443	8.08356
STORAGE TIME	2		12	7.199500	1.349654	0.389612	6.341971	8.05703
STORAGE TIME	5		12	7.900750	0.735680	0.212372	7.433321	8.36818
STORAGE TIME	7		12	9.069500	0.787340	0.227286	8.569248	9.56975
DOSE*STORAGE TIME	0	0	3	8.602000	0.323894	0.187000	7.797404	9.40660
DOSE*STORAGE TIME	0	2	3	8.415000	0.971681	0.561000	6.001212	10.82879
DOSE*STORAGE TIME	0	5	3	7.106000	0.323894	0.187000	6.301404	7.91060
DOSE*STORAGE TIME	0	7	3	9.350000	0.323894	0.187000	8.545404	10.15460
DOSE*STORAGE TIME	2.5	0	3	6.171000	0.000000	0.000000	6.171000	6.17100
DOSE*STORAGE TIME	2.5	2	3	7.667000	0.323894	0.187000	6.862404	8.47160
DOSE*STORAGE TIME	2.5	5	3	8.228000	0.323894	0.187000	7.423404	9.03260
DOSE*STORAGE TIME	2.5	7	3	8.976000	0.000000	0.000000	8.976000	8.97600
DOSE*STORAGE TIME	5	0	3	8.415000	0.000000	0.000000	8.415000	8.41500
DOSE*STORAGE TIME	5	2	3	7.480000	0.323894	0.187000	6.675404	8.28460
DOSE*STORAGE TIME	5	5	3	8.602000	0.647787	0.374000	6.992808	10.21119
DOSE*STORAGE TIME	5	7	3	9.911000	0.323894	0.187000	9.106404	10.71560
DOSE*STORAGE TIME	7.5	0	3	5.610000	0.000000	0.000000	5.610000	5.61000
DOSE*STORAGE TIME	7.5	2	3	5.236000	0.647787	0.374000	3.626808	6.84519
DOSE*STORAGE TIME	7.5	5	3	7.667000	0.647787	0.374000	6.057808	9.27619
DOSE*STORAGE TIME	7.5	7	3	8.041000	0.647787	0.374000	6.431808	9.65019

d. Summary of the Effect of gamma irradiation and storage on the physicochemical quality of smoked guinea fowl meat at 3 ±1 °C

Parameter	Doses (kGy)	Week 0	Week 2	Week 5	Week 7
pH	0	7.19 ±0.000^{h}	7.05±0.010^{de}	7.02±0.020^{bc}	7.15±0.005^{g}
	2.5	7.07±0.010^{ef}	7.01±0.005^{b}	7.16±0.000^{g}	7.30±0.017^{i}
	5.0	6.99±0.020^{a}	7.04±0.005^{cd}	7.08±0.010^{g}	7.15±0.011^{f}
	7.5	7.21±0.005^{h}	7.15±0.000^{g}	7.05±0.026^{de}	7.19±0.006^{h}
TTA (% acetic acid)	0	0.473±0.006^{ef}	0.447±0.021^{de}	0.467±0.006^{ef}	0.610±0.000^{k}
	2.5	0.413±0.006^{cd}	0.357±0.011^{ab}	0.487±0.011^{fg}	0.530±0.010^{hi}
	5.0	0.333±0.015^{a}	0.363±0.045^{ab}	0.567±0.032^{ij}	0.573±0.049^{jk}
	7.5	0.523±0.030^{gh}	0.353±0.011^{ab}	0.387±0.023^{bc}	0.493±0.015^{fgh}
Acid Value (mg/g)	0	8.602±0.324^{fgh}	8.415±0.972^{efg}	7.106±0.324^{c}	9.350±0.324^{hi}
	2.5	6.171±0.000^{b}	7.667±0.324^{cde}	8.228±0.324^{defg}	8.976±0.00^{gh}
	5.0	8.415±0.000^{efg}	7.480±0.324^{cd}	8.602±0.648^{fgh}	9.911±0.324^{i}
	7.5	5.610±0.000^{ab}	5.236±0.648^{a}	7.667±0.648^{cde}	8.041±0.648^{def}

Means ± Standard deviations with different superscripts differ significantly ($P \leq 0.05$).

Appendix 5

a. Analysis of variance table for mean scores of sensory attributes of smoked irradiated guinea fowl meat (Hedonic test)

ANOVA table for Aroma

	Univariate Tests of Significance for Aroma (Spreadsheet5 Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	6630.625	1	6630.625	2352.054	0.000000
Month	0.025	1	0.025	0.009	0.925097
Dose	17.825	3	5.942	2.108	0.101594
Month*Dose	3.025	3	1.008	0.358	0.783649
Error	428.500	152	2.819		

	Descriptive Statistics (Spreadsheet56)							
Effect	Level of Factor	Level of Factor	N	Aroma Mean	Aroma Std.Dev.	Aroma Std.Err	Aroma -95.00%	Aroma +95.00%
Total			160	6.437500	1.681148	0.132906	6.175010	6.699990
Month	1		80	6.425000	1.953737	0.218434	5.990217	6.859783
Month	2		80	6.450000	1.367803	0.152925	6.145610	6.754390
Dose	7.5		40	6.825000	1.430214	0.226137	6.367595	7.282405
Dose	5		40	5.950000	1.866712	0.295153	5.352996	6.547004
Dose	2.5		40	6.650000	1.424151	0.225178	6.194535	7.105465
Dose	0		40	6.325000	1.872712	0.296102	5.726078	6.923922
Month*Dose	1	7.5	20	7.050000	1.571958	0.351501	6.314301	7.785699
Month*Dose	1	5	20	5.850000	2.368099	0.529523	4.741695	6.958305
Month*Dose	1	2.5	20	6.550000	1.538112	0.343932	5.830141	7.269859
Month*Dose	1	0	20	6.250000	2.149051	0.480542	5.244213	7.255787
Month*Dose	2	7.5	20	6.600000	1.273206	0.284697	6.004121	7.195879
Month*Dose	2	5	20	6.050000	1.234376	0.276015	5.472294	6.627706
Month*Dose	2	2.5	20	6.750000	1.332785	0.298020	6.126237	7.373763
Month*Dose	2	0	20	6.400000	1.602629	0.358359	5.649946	7.150054

ANOVA table for colour

Effect	Univariate Tests of Significance for Colour (Spreadsheet5 Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	6617.756	1	6617.756	2530.882	0.000000
Month	18.906	1	18.906	7.230	0.007968
Dose	0.269	3	0.090	0.034	0.991465
Month*Dose	10.619	3	3.540	1.354	0.259224
Error	397.450	152	2.615		

Effect	Descriptive Statistics (Spreadsheet56) Level of Factor	Level of Factor	N	Colour Mean	Colour Std.Dev.	Colour Std.Err	Colour -95.00%	Colour +95.00%
Total			160	6.431250	1.639228	0.129592	6.175306	6.687194
Month	1		80	6.775000	1.574922	0.176082	6.424518	7.125482
Month	2		80	6.087500	1.639649	0.183318	5.722614	6.452386
Dose	7.5		40	6.500000	1.395965	0.220721	6.053549	6.946451
Dose	5		40	6.400000	2.085358	0.329724	5.733070	7.066930
Dose	2.5		40	6.400000	1.428645	0.225889	5.943097	6.856903
Dose	0		40	6.425000	1.615430	0.255422	5.908360	6.941640
Month*Dose	1	7.5	20	7.250000	0.966546	0.216126	6.797643	7.702357
Month*Dose	1	5	20	6.450000	2.064104	0.461548	5.483969	7.416031
Month*Dose	1	2.5	20	6.750000	1.371707	0.306723	6.108022	7.391978
Month*Dose	1	0	20	6.650000	1.694418	0.378883	5.856988	7.443012
Month*Dose	2	7.5	20	5.750000	1.371707	0.306723	5.108022	6.391978
Month*Dose	2	5	20	6.350000	2.158825	0.482728	5.339639	7.360361
Month*Dose	2	2.5	20	6.050000	1.431782	0.320156	5.379905	6.720095
Month*Dose	2	0	20	6.200000	1.542384	0.344887	5.478142	6.921858

ANOVA table for tenderness

Effect	Univariate Tests of Significance for Tenderness (Spreadsheet Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	6969.600	1	6969.600	2485.056	0.000000
Month	1.225	1	1.225	0.437	0.509680
Dose	6.350	3	2.117	0.755	0.521235
Month*Dose	2.525	3	0.842	0.300	0.825285
Error	426.300	152	2.805		

Effect	Descriptive Statistics (Spreadsheet56)							
	Level of Factor	Level of Factor	N	Tenderness Mean	Tenderness Std.Dev.	Tenderness Std.Err	Tenderness -95.00%	Tenderness +95.00%
Total			160	6.600000	1.656700	0.130974	6.341328	6.858672
Month	1		80	6.687500	1.783140	0.199361	6.290682	7.084318
Month	2		80	6.512500	1.526092	0.170622	6.172885	6.852115
Dose	7.5		40	6.925000	1.542351	0.243867	6.431732	7.418268
Dose	5		40	6.425000	1.781493	0.281679	5.855251	6.994749
Dose	2.5		40	6.450000	1.708951	0.270209	5.903451	6.996549
Dose	0		40	6.600000	1.598076	0.252678	6.088911	7.111089
Month*Dose	1	7.5	20	7.200000	1.399248	0.312881	6.545132	7.854868
Month*Dose	1	5	20	6.550000	2.012461	0.450000	5.608139	7.491861
Month*Dose	1	2.5	20	6.450000	1.848897	0.413426	5.584689	7.315311
Month*Dose	1	0	20	6.550000	1.848897	0.413426	5.684689	7.415311
Month*Dose	2	7.5	20	6.650000	1.663066	0.371873	5.871661	7.428339
Month*Dose	2	5	20	6.300000	1.559352	0.348682	5.570201	7.029799
Month*Dose	2	2.5	20	6.450000	1.605091	0.358909	5.698794	7.201206
Month*Dose	2	0	20	6.650000	1.348488	0.301531	6.018888	7.281112

ANOVA table for Taste

Effect	Univariate Tests of Significance for Taste (Spreadsheet Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	7330.556	1	7330.556	2616.519	0.000000
Month	1.806	1	1.806	0.645	0.423264
Dose	10.069	3	3.356	1.198	0.312597
Month*Dose	22.719	3	7.573	2.703	0.047567
Error	425.850	152	2.802		

Effect	Descriptive Statistics (Spreadsheet56)							
	Level of Factor	Level of Factor	N	Taste Mean	Taste Std.Dev.	Taste Std.Err	Taste -95.00%	Taste +95.00%
Total			160	6.768750	1.701726	0.134533	6.503047	7.034453
Month	1		80	6.875000	1.738434	0.194363	6.488131	7.261869
Month	2		80	6.662500	1.668348	0.186527	6.291227	7.033773
Dose	7.5		40	6.800000	1.842518	0.291328	6.210734	7.389266
Dose	5		40	6.350000	1.747526	0.276308	5.791114	6.908886
Dose	2.5		40	6.975000	1.290746	0.204085	6.562199	7.387801
Dose	0		40	6.950000	1.852926	0.292973	6.357406	7.542594
Month*Dose	1	7.5	20	7.300000	1.490320	0.333246	6.602509	7.997491
Month*Dose	1	5	20	6.750000	1.551739	0.346979	6.023764	7.476236
Month*Dose	1	2.5	20	6.950000	1.431782	0.320156	6.279905	7.620095
Month*Dose	1	0	20	6.500000	2.350812	0.525657	5.399786	7.600214
Month*Dose	2	7.5	20	6.300000	2.054520	0.459405	5.338455	7.261545
Month*Dose	2	5	20	5.950000	1.877148	0.419743	5.071468	6.828532
Month*Dose	2	2.5	20	7.000000	1.169795	0.261574	6.452519	7.547481
Month*Dose	2	0	20	7.400000	1.046297	0.233959	6.910318	7.889682

ANOVA table for overall acceptability

Effect	Univariate Tests of Significance for Overall acceptability (Spreadsheet Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	6884.064	1	6884.064	4554.171	0.000000
Month	2.756	1	2.756	1.823	0.178916
Dose	4.895	3	1.632	1.080	0.359652
Month*Dose	5.897	3	1.966	1.300	0.276474
Error	229.762	152	1.512		

Effect	Descriptive Statistics (Spreadsheet56) Level of Factor	Level of Factor	N	Overall acceptability Mean	Overall acceptability Std.Dev.	Overall acceptability Std.Err	Overall acceptability -95.00%	Overall acceptability +95.00%
Total			160	6.559375	1.237036	0.097796	6.366228	6.752522
Month	1		80	6.690625	1.420490	0.158816	6.374511	7.006739
Month	2		80	6.428125	1.013511	0.113314	6.202579	6.653671
Dose	7.5		40	6.762500	1.136445	0.179688	6.399047	7.125953
Dose	5		40	6.281250	1.442473	0.228075	5.819925	6.742575
Dose	2.5		40	6.618750	0.999980	0.158111	6.298941	6.938559
Dose	0		40	6.575000	1.319479	0.208628	6.153010	6.996990
Month*Dose	1	7.5	20	7.200000	1.034281	0.231272	6.715942	7.684058
Month*Dose	1	5	20	6.400000	1.645168	0.367871	5.630038	7.169962
Month*Dose	1	2.5	20	6.675000	1.172884	0.262265	6.126073	7.223927
Month*Dose	1	0	20	6.487500	1.682876	0.376303	5.699890	7.275110
Month*Dose	2	7.5	20	6.325000	1.085490	0.242723	5.816975	6.833025
Month*Dose	2	5	20	6.162500	1.238832	0.277011	5.582709	6.742291
Month*Dose	2	2.5	20	6.562500	0.818676	0.183062	6.179348	6.945652
Month*Dose	2	0	20	6.662500	0.851759	0.190459	6.263865	7.061135

Appendix 5

QUESTIONNAIRE FOR THE EVALUATION OF SMOKED GUINEA FOWL

Name

……

Date

……..

HEDONIC TEST

Test product: **Smoked guinea fowl (Akonfem)**

You are presented with four samples of smoked guinea fowl as labelled below. Please observe the samples and assign scores based on your preference (like or dislike) using the 9-point hedonic scale.

9- Like extremely	6- Like slightly	3- Dislike moderately
8- Like very much	5- Neither like nor dislike	2- Dislike very much
7- Like moderately	4- Dislike slightly	1- Dislike extremely

Sample Code	**263**	**562**	**864**	**434**
Aroma				
Colour				
Tenderness				
Taste				

General acceptability:

Among all samples which one do you like best and please provide reason:
