UNIVERSITÁ DEGLI STUDI DEL MOLISE

Department of Agricultural, Environmental and Food Sciences

International PhD Course in:

AGRICULTURE, TECHNOLOGY AND BIOTECHNOLOGY

(CURRICULUM: ANIMAL WELFARE, BIOTECHNOLOGY AND QUALITY OF ANIMAL PRODUCTION)

(CYCLE XXXI)

Related disciplinary scientific section: AGR/19 (Zootecnica Speciale)

PhD Thesis

Effect of intramuscular vitamin E injection on performance, meat quality and food safety of Cobb 500 broiler chickens reared under Tropical climatic conditions

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DECLARATION

I ACAYE Ongwech, Registration number 155849, of the University of Molise (UNIMOL) – Italy, do solemnly declare that this was my own original work and that it has never before been presented for any award whatsoever in any University or Institution of higher learning worldwide. The production of this work has been under the close guidance and supervision of my supervisors named below.

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DEDICATION

To my late responsible parents who produced and struggled to raise me to the level when the good Lord eventually called both of you in succession, even before the struggle was halfway accomplished. I am very convinced he did that for a purpose; otherwise I would have loved to see both of you witnessing this later part of the struggle. However, he is the Lord and he is good all the time. I pray he continues to keep the souls of both of you, Absalom Ongwech and Yona Adong in high regards forever and ever.

AMEN.

ACKNOWLEDGEMENT

I feel fully indebted to:

My supervisor Professor Giuseppe Maiorano who has been more than a Supervisor but rather a "father" to me away from home. His patience, tolerance, commitment, professional, humane attitude and conduct made me feel at home away from home. May God continue to bless you Professor!

My supervisor Dr Siria Tarvaniello who gave me an inestimable amount of technical, material and social support throughout the entire research process and my stay in Italy. Only the Good Lord can thank you more for such a commitment.

My supervisor Professor Archileo N. Kaaya of Makerere University, who ensured my training at Makerere Universiy went on under appropriate environment, close supervision and unimpeded. Professor, God should continue to guide you to guide others in a similar manner.

The Vice Chancelor and the Director, Institute of Research and Graduate Studies, Gulu University, for their contributions and for giving me study leave with pay for the entire study period that made life possible for my family even when I was away.

Technicians; Okalany Emmanuel of Makerere University and Kifuse Peter of Chemipha Research Laboratory – Kampala, for the technical advice and support accorded to me during the research, more especially during chemical analyses.

All the academic and non-academic staff of the Department of Agricultural Environmental and Food Sciences of the University of Molise, my course mates and friends within and outside Italy for the knowledge, cooperation and support extended to me throughout my academic pursuit of the course at the University.

My wife Joyce, children, other family members, friends and inlaws for their endless encouragement, prayers and support and for taking good care of the family while I was away.

"May God the Almighty listen to my prayers and bless all those persons abundantly"

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LIST OF ABBREVIATIONS AND ACRONYMS

AKR	Aldo keto reductase
α-TTP	Alpha tocopherol transfer protein
BaP	Benzo(a) pyrene
BbF	Benzo(b)fluoranthene
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxy toluene
BkF	Benzo(k) fluoranthene
BgP	Benzo(g,h,i) perylene
BPDE	Benzo(a) pyrene diol-epoxide
CO	Carbonmonoxide
COMb	Carboxymyoglobin
DaA	Dibenzo (a, h) anthracene
DB	Data Bank
DD	Dehydrodiol dehydrogenase
DeoxyMb	Deoxymyoglobin
DHA	Docosahexaenoic acid
DOC	Day old chick
DPA	Docosapentaenoic acid
EDTA	Ethylene diamine tetra acetic acid
EFAs	Essential fatty acids
EPA	Eicosapentaenoic fatty acid
EU	European Union
FA	Fatty acid
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed Convertion Ratio
GC	Gas chromatography
GLM	General Linear Model
Glu	Glutamine
Gly	Glycine
GSH-Px	Glutathione peroxidase
HDL	High-density lipoprotein
HLP	Hydroxylysylpyridinoline
HMGR	3-hydroxy-3-methyl glutarylCoA reductase enzyme
HPAI	Highly Pathogenic Avian Influenza
HS92	Harmonised System (A commodity coding system formulated in 1992)
IcP	Indeno(1,2,3-cd)pyrene
Ile	Isoleucine
IMC	Intramuscular collagen
IMFNB	Institute of Medicine Food and Nutrition Board
LA	Linoleic acid
LDL	Low-density lipoprotein
Leu’	Leucine
LOD	Limit of Detection
LOQ	Limit of Quantitation
LPL	Lipoprotein lipase
Lys	Lysine
Mb	Myoglobin
MDA	Malondialdehyde

MEG	Marktbilanz: Eier und Geflügel
Met	Methionine
MetMb	Metmyoglobin
Mg	Magnesium
MRP	Maillard reaction products
MUFAs	Monounsaturated
NAGRIC	National Animal Genetic Resources
NCBI	National Center for Biotechnology Information.
NRC	National Research Council
OEC	Observatory of Economic Complexity
OxyMb	Oxymyoglobin
PCI	Product Complexity Index
PFTBA	Perfluorotributylamine
PG	Propyl gallate
PGE2	Prostaglandin E2
Phe	Phenylalanine
PLA2	Phospholipase A2
Pro	Proline
PSE	Pale, Soft, Exudative
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SAARI	Serere Agricultural and Animal Research Institute
Se	Selenium
SEM	Standard Error Means
Ser	Serine
SFAs	Saturated fatty acids
SOD	Super-Oxide Dismutase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric Acid Reactive Substances
TBHQ	Tertiary Butyl Hydroquinone
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalency
USA	United States of America
USEPA	United States Environmental Protection Agency
VLDL	Very Low Density Lipoproteins
VOD	Vegetable Oil Distillates
WHC	Water-Holding Capacity
WHO	World Health Organization
WOF	Warmed-over flavor

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ABSTRACT

An important issue in poultry farming is the increased susceptibility of the animals to oxidative stress related to the increasing genetic selection toward larger breast muscles, increased body weights, and faster growth rates. Vitamin E as a potent natural antioxidant, is a well-established micro-nutrient used in animal nutrition to sustain animal health and production, and to reduce the oxidative processes in meat. Studies have also reported inhibitory effects of vitamin E on formation of carcinogenic compounds during meat processing. However, these tests mainly involved the less standardised inclusion of vitamin E in animal feeds or the topical application of vitamin E for marination of meat. The present study, therefore, aimed at assessing the effect of intramuscular vitamin E injection on growth performance, meat quality and safety of processed meat of Cobb 500 broiler chickens reared under tropical conditions in Uganda. The study was carried out in a small chicken farm in Uganda. Thirty, 28d old Cobb 500 chicks were randomly assigned to 3 treatment groups (5 replications/group), which were intramuscularly injected on left pectoral muscle with 0.25 mL (12.5 IU) (T1) and 0.5 mL (25 IU) (T2) of *dl*–α–tocopheryl acetate (50 mg/mL), and 0.5 mL of physiological saline (C). At 42d, birds were individually weighed, slaughtered and the pectoral muscle (PM) was removed for the analyses regarding physico-chemical properties, oxidative stability and detection and quantification of carcinogenic compounds. Data were analyzed by one-way ANOVA. Data for concentration of polycyclic aromatic hydrocarbons (PAHs) that did not meet the assumptions for ANOVA were analyzed using the Kruskal Wallis H test. The results indicate that i.m. vitamin E injection did not have significant effect on the final live weights of the birds. However, carcass yield was higher ($P < 0.01$) in T2 (71%) and T1 (69%) groups compared with C group (64%). The weights and yields of the main commercial cuts were similar among groups. Ultimate pH of PM was not significantly affected by vitamin E treatment; while higher doses of vitamin E (T2) improved the water

holding capacity of PM ($P<0.01$). Total lipids, ranging from 2.13% to 2.25%, were similar among groups. In general, vitamin E treatment improved the oxidative stability of PM; the levels of TBARS were lower ($P < 0.05$) in T2 (0.037 mg MDA/kg) than in C group (0.046 mg MD/kg). The mean concentrations (μg/kg) of PAHs formed in grilled meat were in the range: 0.30 – 1.55 for Benzo(a) pyrene, 0.37 – 2.54 for Benzo(b) fluoranthene, 0.80 – 1.19 for Benzo(g,h,i) perylene, 0.89 – 1.97 for Benzo(k) fluoranthene, 0.94 for Dibenzo(a, h) anthracene and 1.18 – 1.57 for Indeno (1,2,3-cd) pyrene. This research has shown that whereas there appears to be no significant effect of the treatment with vitamin E on formation of PAHs during grilling of broiler PM, an apparent trend towards inhibition of the formation of these carcinogenic compounds appeared to have emerged. This was evidenced by the fact that more of the compounds were detected in C as opposed to the vitamin E injected groups. The results from the Toxic Equivalent computation also revealed that the concentrations of the compounds detected were within the tolerable limit set by EU Regulation 835/2011 still in force. It should be noted that this attempt to investigate the inhibitory effect of i.m vitamin E injection on the formation of carcinogenic compounds in broilers has been probably the first; therefore, further research is required to obtain more information, regarding also the adequate dose of vitamin E to be injected.

RIASSUNTO

L’intensa selezione genetica ha consentito negli anni di ottenere polli sempre più pesanti nel minor tempo possibile, rendendo gli animali maggiormente suscettibili allo stress ossidativo. La vitamina E, un potente antiossidante naturale, è un micronutriente utilizzato nell'alimentazione animale per migliorare lo stato di salute e la qualità delle produzioni, nonché per ridurre i processi ossidativi nella carne. Recenti studi, hanno dimostrato come la vitamina E, attraverso la marinatura della carne, sia in grado di inibire la formazione di composti cancerogeni in seguito a cottura ad elevate temperature. Il presente lavoro di tesi ha inteso valutare gli effetti della somministrazione di vitamina E per via intramuscolare sulle performance di crescita, sulle caratteristiche qualitative della carne e sulla formazione di sostanze cancerogene nella carne di polli Cobb 500 allevati in condizioni di clima tropicale in Uganda. Al 28° giorno d’età, 30 polli Cobb 500 sono stati suddivisi a random in 3 gruppi sperimentali (5 replicazioni/gruppo) trattati per via intramuscolare (muscolo pettorale sinistro) con: 0.25 mL (12.5 UI) (T1) e 0.50 mL (25 UI) (T2) di *dl*- α-tocopheril acetato (50 mg/mL), e 0.50 mL di soluzione fisiologica (C). Al 42° giorno d’età, i polli sono stati pesati, macellati e il muscolo pettorale è stato prelevato per le analisi riguardanti le proprietà fisico-chimiche, stabilità ossidativa e formazione di sostanze cancerogene. I dati ottenuti sono stati analizzati mediante ANOVA ad una via. I dati riguardanti la concentrazione delle idrocarburi policiclici aromatici (IPA) non rientranti nelle assunzioni dell’ANOVA sono stati analizzati mediante test di Kruskal Wallis. Il trattamento non ha avuto alcun effetto ($P > 0,05$) sul peso finale degli animali; tuttavia, i polli dei gruppi trattati hanno mostrato rese in carcasse maggiori (T2: 71% e T1: 69%; $P < 0,01$) rispetto al gruppo C (64%). Il peso e la resa dei principali tagli commerciali sono risultati simili tra i gruppi sperimentali. Il pH ultimo non è stato influenzato dal trattamento; mentre, la dose più alta di vitamina E (T2) ha determinato un aumento della capacità di ritenzione idrica del petto ($P < 0,01$). Il contenuto di lipidi totali (2,13 - 2,25%) è

risultato simile tra i gruppi sperimentali. I livelli di TBARS (mgMDA/kg) sono risultati inferiori ($P<0,05$) nel gruppo T2 (0,037) rispetto al gruppo C (0,046). Le concentrazioni medie (µg/kg) di IPA formatesi durante la cottura alla griglia della carne rientravano nei seguenti range: 0,30 – 1,55 Benzo(a)pirene, 0,37 – 2,54 Benzo(b)fluorantene, 0,80 – 1,19 Benzo(g,h,i)perilene, 0,89 – 1,97 Benzo(k)fluorantene, 0,94 Dibenzo(a,h)antracene e 1,18 – 1,57 Indeno(1,2,3-cd)pirene. Questa ricerca ha dimostrato che, nonostante il trattamento con vitamina E non abbia avuto alcun effetto statisticamente significativo sulla concentrazione degli IPA, è evidente una tendenziale inibizione della formazione di questi composti in seguito a cottura della carne. Questa assunzione deriva dal fatto che la maggior parte dei composti sono stati rilevati nel gruppo C e non nei gruppi vitamina E. I dati relativi alla tossicità equivalente hanno evidenziato che le concentrazioni dei composti rilevati rientrano nei limiti di legge in vigore (Reg. UE 835/2011). Lo studio sull'effetto dell'iniezione intramuscolare di vitamina E sulla formazione di composti cancerogeni nei polli da carne è stato probabilmente il primo; sono quindi necessarie ulteriori ricerche per ottenere maggiori informazioni, anche riguardo alla dose adeguata di vitamina E da iniettare.

PART I INTRODUCTION AND LITERATURE REVIEW

Chapter 1

POULTRY MEAT: PRODUCTION, CONSUMPTION AND TRADE

1.0 Introduction

The term Poultry refers to domesticated birds kept by humans for production of eggs, for their feathers, meat and organs. Whereas many people normally tend to think of the term as referring to chickens, it is broader and includes other birds like turkeys, geese, ducks, quails, pigeons, ostriches and others (The American Heritage, 2009). Poultry make quite a substantial contribution to food security and nutrition, providing energy, proteins and essential micronutrients to humans. This is possible because of the short production cycles and the ability to convert a wide range of agri-food by-products into meats and eggs for human consumption (Mottet & Tempio, 2017). Poultry is the fastest growing agricultural subsector, especially in developing countries where there is a faster rate of growth in both population and economy than in developed countries. To meet growing demand, world poultry meat production soared from 9 to 120 million tonnes between 1961 and 2016, and egg production shot up from 15 to 81 million tonnes. In 2016, poultry meat represented about 36 percent of global meat production. In the last three decades, world egg production has increased by more than 150 percent. Much of this growth has been in Asia, where production increased almost fourfold (FAO, 2018).

The global human population is expected to reach 9.6 billions in 2050 with 82% expected to come from developing countries as opposed to only 79% in 2000. Further, it is projected that approximately 70% of these people shall be living in urban areas. On the other hand, income is expected to increase by 2% per year over the period to 2050. Consequently, Alexandratos & Bruinsma, (2012) projected that the demand for animal source food could

grow by 70% between 2005 and 2050 with poultry meat expected to have the highest growth of about 121%. The global poultry sector is expected to continue to grow as demands for meat and eggs continue to rise. The fact that poultry can be managed by almost all sectors such as small holders, the poor rural and peri-urban population, and also in large scale intensive production systems accounts for its faster growth rate (Mottet & Tempio, 2017). The average annual growth rate over the last 5 decades was 5% while it was only 1.5% for beef, 3.1% for pork and 1.7% for small ruminants'meat (Alexandratos & Bruinsma, 2012).

World meat consumption according to OECD and FAO (2014) projection is expected to average 36.3 kg per person per year in retail weight by 2023; an increase of 2.4 kg compared with the figure for 2013. Some 72% of the increased consumption will come from poultry. The report further states that the high feed conversion ratio, short production cycle and simple production process makes poultry the low cost alternative which consumers in developing countries demand first as their income levels rise. Summarily, the current and expected increase in poultry production and consumption are primarily linked to key factors of population growth, urbanization, improvements in incomes, lower costs of production, the lower poultry meat prices relative to those for competitive meats, dietary preference, general acceptability across religions and cultures as well as consumer sentiments related to health, the environment and convenience (Marangoni et al., 2015; Mutryn et al., 2015).

1.1 Poultry meat production

1.1.1 Global production

Global poultry meat production has been on the rise and increased from approximately 54.2 million tonnes in 1995 to 107.0 million tonnes in 2013 (FAO, 2014); reached a high of about 116 million tonnes in 2016 (GLEAM 2, 2016) and is expected to increase by over 181 million tonnes by 2050 (Alexandratos & Bruinsma, 2012). Worldwide, no other agricultural product has reached such a remarkable relative growth rate. Among the factors that were

cited as responsible for the poultry sector growth include technological changes in production practices (Mottet & Tempio, 2017). Thus, the move from free-ranging to confined poultry operations dramatically increased the number of birds per farmer, facilitated the substitution of capital for labour, and led to a significant increase in labour productivity. Other factors included advances in breeding to improve animal size, fecundity, growth rate and uniformity, improved disease control and biosecurity measures (Thornton, 2010).

Production has been particularly dynamic in developing countries, especially in East and South East Asia with an annual growth rate in poultry meat production of 7.4% (FAOSTAT, 2016) and also in South America. In 1970, only two of the ten leading countries were developing countries, and only China and Japan, were located in Asia. With a share of almost 31%, the USA dominated this branch of poultry production. In 2005, five of the ten leading countries were developing countries and four were located in Asia. The United States were still in the top position with a share of 22.9%, but China and Brazil now ranked as number two and three. The regional concentration slightly decreased from 68.4% to 66.3% as a result of the growing importance of poultry meat production in several other countries (Windhorst, 2018).

Among countries currently leading in poultry meat production are the United States of America (~20 million tonnes), China (~18 million tonnes), European Union (EU), (~13 million tonnes), Brazil (~13 million tonnes) (Bedford et al., 2017). The US leads in the production due mainly to technological advancement, the ability and also the willingness to purchase the poultry meat. China is second to the U.S., and its production is increasing. While China is a relatively poor country, its population is more than 1 billion, and its people eat chicken whenever it is available. Even small increases in per-capita consumption in China cause large changes in demand for chicken. Brazil, which is the third-largest poultry producer, is a major player in the world poultry market. It has the needed grain production to feed

chickens and has an ample supply of labour to grow and process the birds. It also has access to the same technology found in U.S. plants (Bedford et al., 2017). For the EU that also has the technology and expertise, in 2016, ten countries alone contributed 88.5% of the total tonnage of poultry meat per year within the union (Figure 1.1)

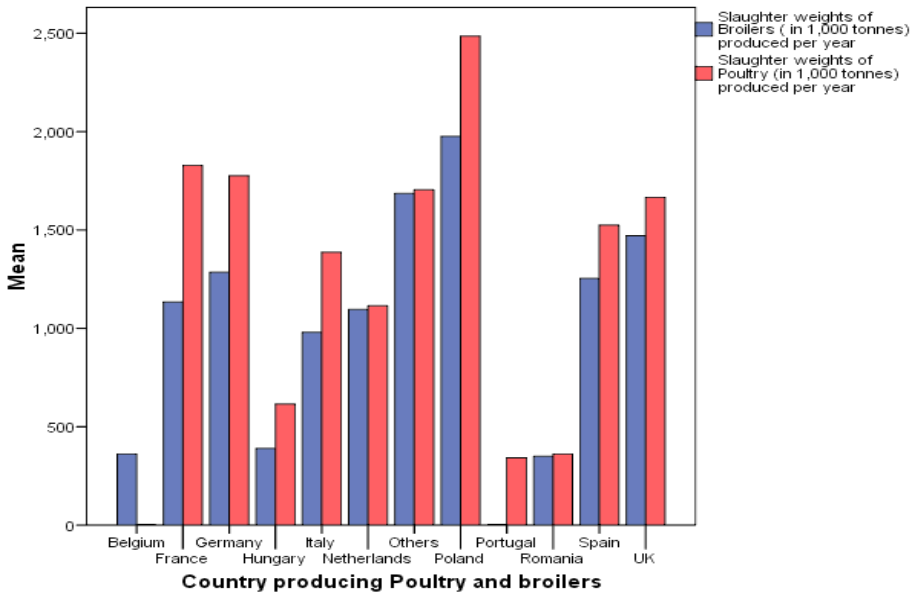


Figure 1. 1 The ten leading EU countries in poultry meat production (2016)
Based on Data supplied by MEG 2017

Figure 1.1 indicates that, whereas Poland maintained its lead in the production of both poultry meat in general and broiler meat in particular, UK surpassed France and Germany when only broiler meat production was considered. Spain also surpassed France and the Netherlands ranked before Italy. Romania that did not feature among the top ten EU poultry meat producing countries, replaced Portugal in tenth position when only broiler meat production was considered. The four leading countries contributed 49.9% broiler meat production while the top four countries (Germany, 19.4%; France, 17.7%; Poland, 17.4% and Italy, 15.6%) contributed 70.1% of turkey meat production in 2016 (Windhorst, 2018).

Between 2012 and 2016, EU poultry meat production increased by almost 1.8 million tonnes or 13.8%. To this growth, broiler meat contributed 1.9 million tonnes. The absolute growth of this meat type was higher than the increase of total poultry meat because the production of other poultry meat types decreased. Turkey meat production grew by 6.5%

while duck meat remained on a more or less constant level (Table 1.1). In 2016, broiler meat alone constituted 81%, turkey 14%, duck 3% and other poultry types 2% in the EU countries (Windhorst, 2018).

Table 1. 1 Trends in the EU poultry meat production (2012 - 2016) by meat type

Meat type	2012	2014	2016	Change
Broiler meat	10,078	10,805	11,964	+ 18.7
Turkey meat	1,981	1,944	2,109	+ 8.5
Duck meat	509	532	510	+ 0.2
Others	450	446	225	- 50.0

Source: MEG 2017 as reported in Windhorst,(2018). (Data in 1,000 t)

Whereas it still remains the fastest growing agricultural subsector, poultry meat production is projected to increase at a slower rate than in the past decades. By 2050, its annual growth rate is estimated to reach 1.8% at global level, and 2.4% in developing countries (Mottet & Tempio, 2017).

1.1.2 Production in Uganda

In Uganda especially in the rural areas, a large number of people do not have an adequate and regular intake of animal proteins. According to a report of the Livestock Services Project by the Agricultural Secretariat of the Bank of Uganda (1989), animal protein formed only about 3% of the diet of the population. In rural areas, even in homes where cattle are available, tradition, and the fear of depleting the stock have limited the number and rate of slaughter for meat consumption, while in urban areas, the socio-economic problems and the high cost of meat have denied many families access to this source of animal proteins (Ojok, 1993). Consequently, many people in both the rural and urban areas have resorted to poultry production; which provides a relatively cheaper and quicker access to animal proteins through the production of eggs and table birds. The increased production of the birds in addition improves the economic status of the farmers (Ojok, 1993).

Poultry meat production in Uganda by 2014 stood at 54,868 metric tonnes and is projected to increase to 63,647 metric tonnes in 2020 with the greatest percentage comprising chickens (MAAIF, 2016 unpublished). Over 86% of these are chicken breeds that are indigenous varieties in village flocks that are low-maintenance scavengers living in close proximity to their owners in small rural communities under the backyard system (Kugonza et al., 2008). These local chickens are predominant in villages despite the introduction of exotic and crossbred types, because farmers have not been able to afford the high input requirements of introduced breeds. However, cross breeding of local chickens with exotic chicken breeds has also been taking place and it is common to find mixed breeds as a result of target improvement programmes by different organisations such as the SAARI chicken breeding project or on individual level (Sorensen & Ssewanyana, 2003).

It is worth noting that the genetic resource base of indigenous poultry in Uganda is rich and has great potential for genetic improvement and diversification to produce well adapted breeds that can be used in genetic improvement programmes for tailored production systems. However, information on the genetic make-up of the indigenous poultry in Uganda is very limited. Although efforts for genetic preservation and biodiversity was planned by the National Animal Genetic Resources and Data Bank (NAGRIC & DB), this has not yet been fully implemented (Ssewannyana et al., 2003). On the other hand, the continued cross-breeding programmes in rural poultry which do not consider gene preservation aspects may lead to the erosion of the indigenous germplasm (Byarugaba, 2007).

Village flocks range from 5 to 20 birds per household, with an overall national average of 12 (Kugonza et al., 2008) although there is wide regional variation. The average egg production reaches 40 eggs per hen per year while the average rooster weight at the time of sale or consumption ranges from 1- 2 kg. This is suitable for the rural consumers who neither desire nor can afford larger birds. The birds exhibit a wide phenotypic variability in plumage,

shank, eye, earlobe, comb, feather distribution and body size (Byarugaba & Katunguka-Rwakishaya, 2002; Ssewannyana et al., 2003) (Appendix 3). The local breeds and the backyard rearing systems have a number of advantages and limitations as shown in Table 1.2

Table 1. 2 Advantages and limitations of backyard rearing and the local chicken breeds

Advantages of the backyard local chickens	**Limitations of backyard local chickens**
• They are self-sustaining i.e. can raise their own replacement stock.	• They have low growth rate.
• They are hardy birds that can survive hard conditions.	• They produce fewer small sized eggs and comparatively little meat.
• Management requirements are not critical as those of commercial exotic breeds.	• People keep them without commercial intentions.
• They are immune to some diseases and parasites.	• They have been neglected by breeders/ scientists despite their potential.
• Their products fetch more money than those from exotic birds.	• They are prone to predators when free ranged.
• Their meat and eggs are more nutritious.	• Backyard chickens poop everywhere and make the compound dirty.
• May help weed and debug compounds.	• May eat up the greenery creating patches.

Practices vary, but the birds are generally either raised by the women and girls of individual families or, in rare cases kept by rural cooperatives (Byarugaba, 2007). Village chickens also fulfill a range of other functions for which it is difficult to assign a monetary value. They are active in pest control, provide manure, are required for special festivals and to meet social obligations; are essential for many traditional ceremonies (for example slaughter for important guests) and traditional and "spiritual" treatment of illnesses and rituals (Byarugaba, 2007).

The remaining 14% are exotic type chickens raised in commercial units close to towns and urban markets. Producers in this production system aim at using the recommended standard practices, such as breed of choice depending on production objectives, appropriate

housing, feeding and health and disease control programmes. The system can be classified into the following categories based on scale of production:

- Small-scale units categorised under FAO Sector 3. Mainly household/family owned farms of 100 – 500 layers or broilers or indigenous chickens.
- Medium-scale units owned by individuals, companies or farmers' groups, with a capacity of between 500 – 5,000 layers or broilers and therefore more specialised in terms of production. This category also includes farms with a small parent breeding stock and hatching facilities. Bio-security levels are low at these units and they would also be categorised as FAO sector 3.
- Large scale units which are mainly breeders with hatching facilities and a capacity of over 5,000. These would be categorised as FAO Sector 2.
- Breeding farms (hatcheries) whose main commercial activity is keeping parent stocks and selling of DOCs. Almost all of them will also be involved in keeping commercial layers or broilers as well (Byarugaba, 2007).

The most common exotic breeds in Uganda include Cobb 500, Cobb 700, Ross 308, Ross 208 and the newly introduced Kuroilers. Cobb 500 is a tetralinear hybrid produced by Cobb Breeding Company from the United Kingdom, being sexable at age of one day after primary remiges' development. It is characterized by a great chest weight, broiler uniformity and increased slaughtering efficiency (Figure 1.2). Compared to Ross 308 hybrid, Cobb 500 is pretentious to breeding conditions and less pretentious to fodder quality, and for that to express the genetic potential there must be provided the microclimate parameters at optimal level (Pasca et al., 2008). The name for the Cobb 500 actually came from a remark by Robert Cobb Junior who felt the new bird would be five times better than the Cobb 100 that had been the company's mainstay for many years. Lowest feed conversion together with the ability of the Cobb 500 to thrive on lower density amino acid, less expensive feed, excellent growth

rate, broiler uniformity for processing and being a competitive breeder reduces the cost of producing chicken meat without affecting performance. This is what many low income farmers in developing countries advocate for.

Figure 1. 2 Cobb 500 broiler chicken

Source: http://www.cobb-vantress.com/products/cobb-500

1.2 Global Poultry meat consumption

The poultry sector has been the most dynamic meat sub-sector in the last decade, showing the greatest growth of all meat sectors as reflected in world consumption. Poultry meat remains the most produced meat in terms of metric tonnes, lead by the United States of America, China and Brazil. According to a recent FAO report, the poultry sector modestly expanded coming in at 120.5 million metric tonnes in 2017 and fore cast at 122.5 metric tonnes in 2018, representing a change of 1.6% between 2017 and 2018. The FAO report further states that poultry has been the meat with the largest production since 2016. For another year, developing countries (categorized based on GDP, per capita income and industrialization), captured the majority of poultry meat production and consumption in 2017. Although production has slowed, changing pattern of feeding in developing countries continue to increase poultry production world wide. The United States of America, China and

Brazil remain the leading countries in poultry meat production with the 2018 figures for those countries forecast by FAO at 22.35, 17.81 and 13.74 million metric tonnes respectively. The other notable countries are the European union, Russia and Mexico (Figure 1.3)

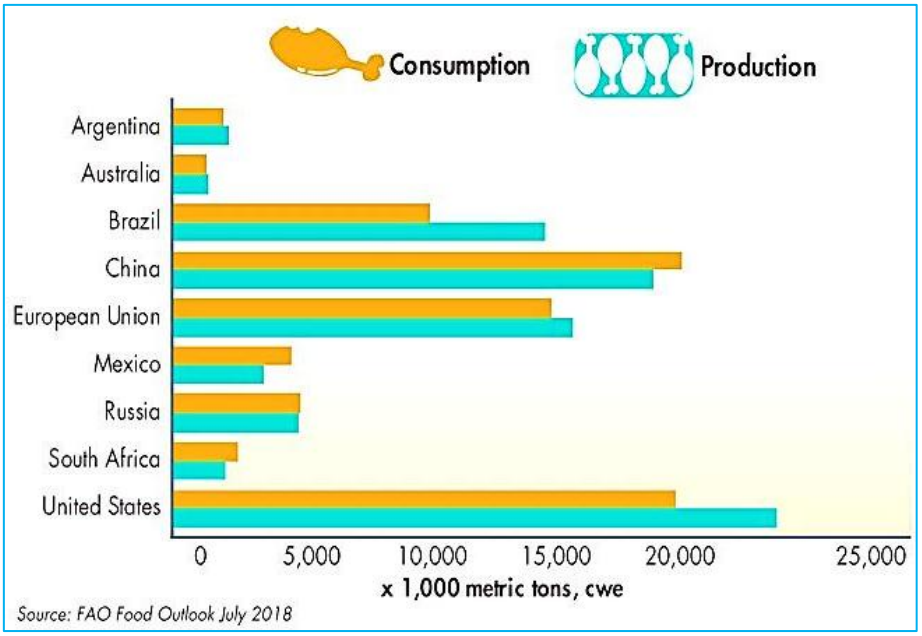


Figure 1. 3 Poultry meat production and consumption in selected countries

The shift in dietary patterns in developing countries have resulted in world meat consumption average of 34.3 kg/person/year over the average base period 2015 -2017 and will continue to grow slowly as population growth slows down by about 1 kg over the next decade to about 35.4 kg/person/year by the end of 2027. Of this consumption, poultry sits at 13.91 kg/person/year for the base period 2015 – 2017, rising only subtly to 14. 70 in 2027 (OECD/FAO, 2017).

The most evident growth of poultry meat consumption was observed in East and Southeast Asia and in Latin America, particularly in China and Brazil. According to the report of OECD/FAO, (2018), the past decade saw strong growth in the global per capita consumption of poultry (+16%), while the per capita consumption of beef and veal decreased by almost 5% between 2008 and 2017. In 2017, the top 10 countries in per capita poultry meat consumption (kg/person/year), according to the report were; Israel (56.93), USA (48.83),

Saudi Arabia (44.67), Australia (44.47), Malaysia (41.69), Brazil (39.90), Peru (38.24), Newzealand (37.86), Argentina (47.51) and Canada (34.89).

On the African continent, the report further states that South Africa (32.49) was ranked 12th while on the lower end of the scale were notably African countries; Sudan (0.00098), Rwanda (0.200), Chad (0.400) and Kenya (0.600). Moreover earlier reports by Narrod & Tiongco, (2012), estimated that consumption of poultry meat in developing countries will increase until 2030 by 3.5% per annum. The per capita income growth in developing countries adds to the consumption of each person. The main impetus for consumption growth will come from developing countries since their per capita income growth rate is expected to be higher. In addition, poorer people tend to spend much of their additional income on food (i.e. they have higher income elasticity of demand for food). For example according to the OECD/FAO, (2016) report, the share of additional income that will be spent on food in China and the United States of America in 2025 is projected to correspond to 3.4% of the income increase in China and only 1.1% of the income increase in the United States of America.

Several researchers have evaluated the factors responsible for meat consumption.The majority of them reported on tastes, prices, product consistency, income levels, household size, education and health consideration as the major factors (Antwi-Boateng et al., 2013; Damisa & Hassan, 2009; Reicks, 2006). These authors also observed that generally demographic variables such as gender, age and religious beliefs significantly affect the attitude of consumers towards the purchase of meat; factors which must be taken into consideration by meat producers and marketers.

1.3 Trade of poultry meat

Poultry meat is the 137th most traded product and the 672nd most complex product out of 1,238, according to the Product Complexity Index (PCI) and is a 4 digit HS92 product (OEC 2017). However as far as the livestock industry is concerned, poultry is the main

product traded, representing 43%, followed by bovine, pig and ovine meat, respectively (FAO, 2014). Poultry meat is traded as raw meat and in the majority of cases as cuts (parts) rather than the whole carcasses; a trade system that makes poultry meat more accessible to the average consumer in low income countries (Josling etal., 2001). Moreover, even in developed countries, consumer eating habits have shifted to poultry breast and thigh meat and, to a lesser extent, drumsticks (Da Silva, 2013). Of late poultry meats are also increasingly being sold in fried, steamed, or roasted forms that are usually packaged "ready-to-eat", and shipped frozen. Consumer demands for these types of prepared food are rapidly increasing especially in developed countries (Nicita, 2008).

According to Nicita, (2008), poultry trade is often subjected to substantial tariffs often on a preferential basis, and non-tariff measures such as veterinary certification, licensing, product characteristic requirements and quotas. These policy instruments help shape bilateral trade flows by limiting imports but may sometimes also favour only determined countries.

According to the FAO, (2018) report, world poultry meat exports reached 13 million tonnes in 2017, up 2.8% from 2016. While Brazil, the United States, the EU and Thailand continued to dominate export markets, most of the increase in shipments in 2017 originated in the United States (+3.1%), Turkey (+33.6%), Thailand (+10%), China (+15%), the Russian Federation (+40.7%) and the EU (+2.5%). The US benefitted from its HPAI-free status and strong import demands from Mexico, China, Cuba, Canada and Angola. The expansion in Thailand was driven by demand for value-added ready-to-eat products in world markets, especially in Japan.

Despite the import bans by key trading partners in response to HPAI outbreaks, EU poultry exports expanded mainly due to the resilience of the sector and the capacity to contain the spread and to identify new markets. In contrast, Brazil's exports slipped by 2 percent in 2017 to 4.3 million tonnes but continued to be the world's largest poultry meat exporter.

Brazil shall continue to benefit from demands in its key Middle East markets due to the ability to adapt to new Saudi Arabian market conditions of non-stun slaughter (USDA, 2018).

The top ten importers of poultry meat and poultry meat products by quantity and by value are shown in Figure 1.4; which indicates that, there is variation in the ranking by quantity imported and by value of the import. When quantity is used for ranking, Netherlands tops the list followed by Germany and Mexico in that order. The other seven countries follow in the order; Hongkong, Belgium, Japan, UK, France, China and Russia. While if the value (in thousands of US $) of the products imported is used as the basis for ranking then Germany tops the list and the rest follow in the order: Netherland > UK > Hongkong > Japan > France >Mexico > China >Belgium >Russia.

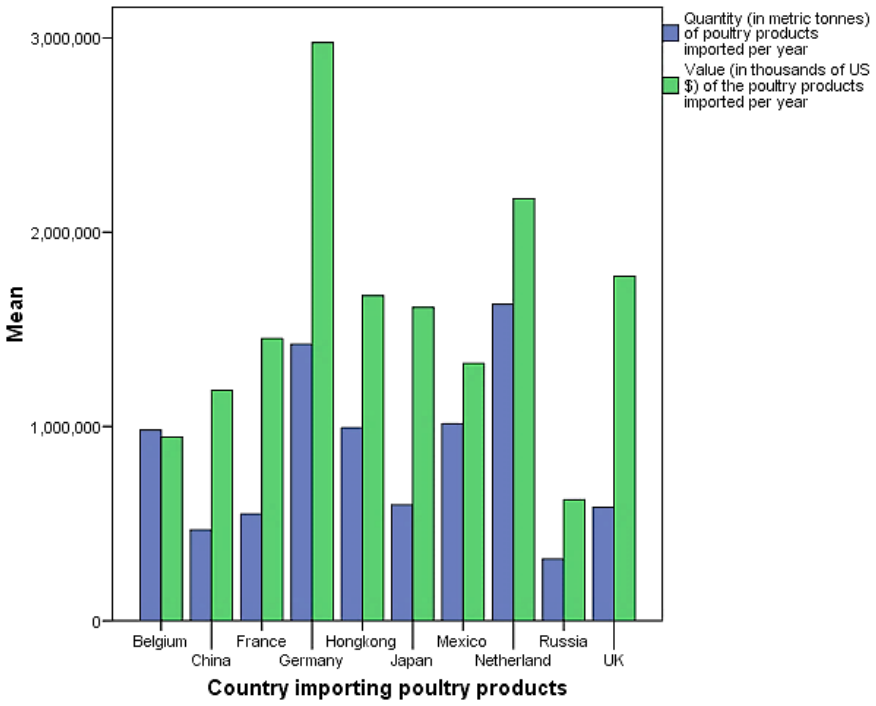


Figure 1. 4 Top ten countries importing poultry products
Based on data from COMTRADE, (United Nations, 2017)

Chapter 2

POULTRY MEAT: QUALITY ATTRIBUTES

2.0 Introduction

The term quality as relates to poultry meat is difficult to define because quality is 'in the eye of the beholder'. For example, someone trying to sell a product might view its quality in terms of how well it sells and how much people are willing to pay for it. However, this definition is incomplete because it does not consider the product's character. Since people only buy what they like, the consumer's perspective of quality is more appropriate. When consumers buy a poultry product, cook and serve it to their families, they expect it to look, taste and feel good in their mouth. If these characteristics do not meet the consumer's expectation, the product is considered to be of lower quality (Julie, 1997). From the point of view of the consumer, meat quality may be categorised based on the following attributes:

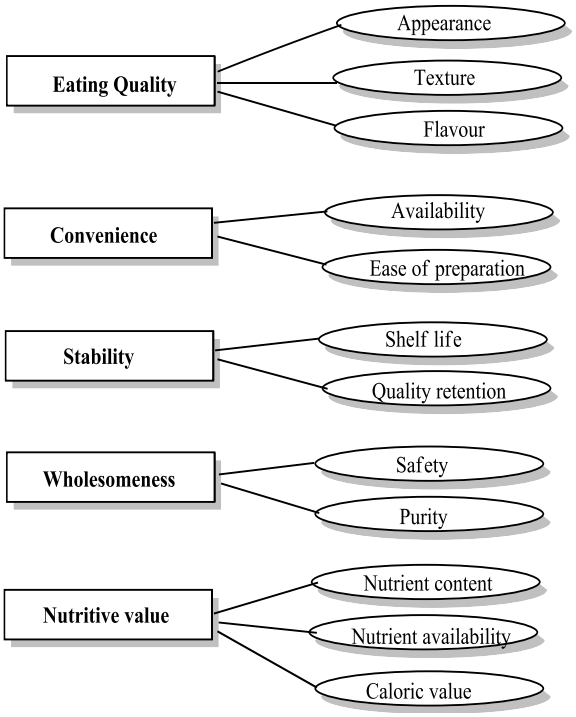


Figure 2. 1 Quality attributes of a food product
Adopted from Erdtsieck, (1989)

2.1 Sensorial and eating quality

A number of meat quality traits are necessary to arouse and sustain interests in the consumer of poultry meat. These traits include those concerned with visual, olfactory and general palatability attractions; and include:

2.1.1 Appearance (Colour)

Appearance is among the most critical judgement criteria consumers use while selecting food items, including poultry products for purchase. Appearance is also critical for the final product evaluation due to its effects on other sensory properties. One of the major contributing components of appearance is colour. Colour has long been used as a yardstick for both freshness of meat as well as the ability of the meat to offer the final product satisfaction (Fletcher, 1999). Poultry meat is sometimes sold with the skin and bone intact and as such, colour is important for the skin, the meat, and the bone. Skin colour is most critical for the marketing of fresh whole birds or parts. Meat colour is most important both for the selection of deboned and skinless raw meat. It is as well a critical factor for the final evaluation of many cooked products. Pink or red appearance of cooked poultry meat is generally associated with undercooking and is highly undesirable. Dark or black bones are also considered to be a defect in fully cooked products. Bone darkening is primarily associated with frozen products prior to cooking. Other visual defects are associated with bruises, haemorrhages, blood pooling and a number of other possible variation factors (AMS, 1995).

Poultry meat colour is affected by factors such as the bird's age, sex, strain, diet, intramuscular fat, meat moisture content, pre-slaughter conditions and processing variables. Colour of meat depends upon the presence of the muscle pigment myoglobin. Differences in the colour intensity of poultry meat can be related to the amount of this pigment that is present in the meat, the chemical state of the pigment, or the way in which light is reflected off the meat. The change in colour intensity can occur in an entire muscle, or it can be limited to a

specific area, such as a bruise or a broken blood vessel. When colour of an entire muscle changes, it is frequently the breast muscle; being the one that accounts for upto about 5% of the live weight; more sensitive to factors that contribute to changes in colour intensity, and because its already light appearance makes small changes in colour more noticeable.

Extreme environmental temperatures or stress due to live handling before processing can cause changes in broiler meat colour; the extent of which is related to the bird's individual response to the conditions and is normally brought about by the activity of the sarcoplasmic myoglobin, (Figure 2.2), which is species specific (Surendranath & Poulson, 2013).

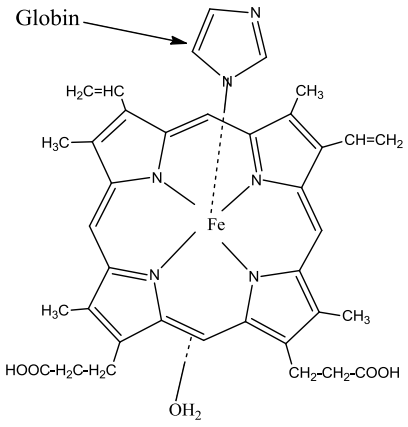


Figure 2. 2 The chemical structure of myoglobin
Adopted from Chaijan, (2008)

Naturally fresh meat can be classified as red or white depending on the concentration and extent of oxygenation of myoglobin in the muscle fibres (Wang et al., 2012). The more myoglobin molecules meat contains the more intense the red colour shall be. Myoglobin content is higher in beef and lower in poultry with lamb and pork having intermediate amounts. The interaction of myoglobin with oxygen and carbonmonoxide, the change in oxidation states of the haeme iron, resulting into the different states of myglobin and hence the different colours of meat during aging or processing was summarised by Mancini & Hunt, (2005) as seen in Figure 2.3.

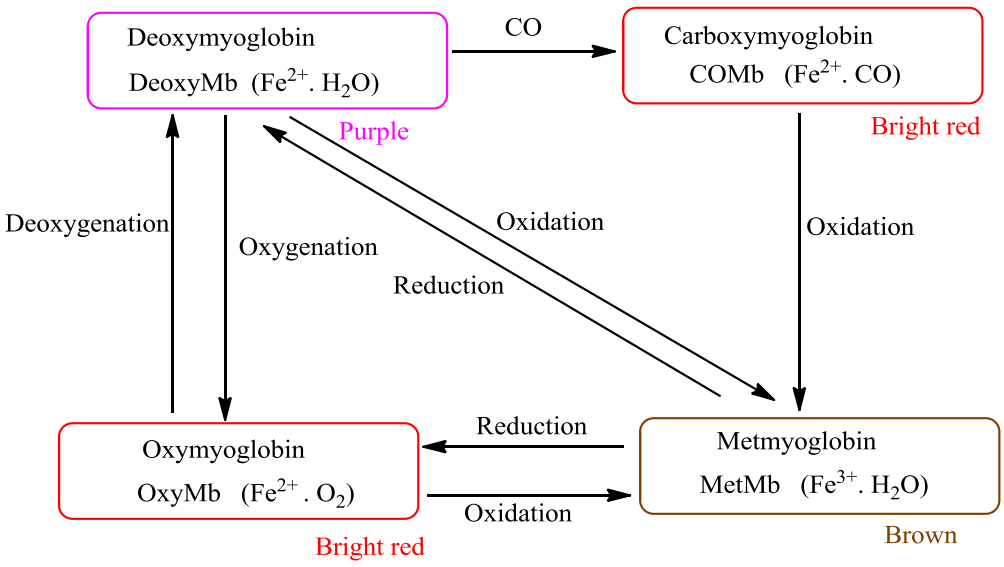


Figure 2. 3 Myoglobin redox forms in fresh meat
From Mancini & Hunt, (2005)

DeoxyMb, OxyMb, and COMb are in a ferrous state. Saturating Mb with oxygen provides attractive cherry-red colour to meat through formation of OxyMb. OxyMb and COMb provide bright cherry-red colour critical to acceptance, and the red colour of these two redox forms is indistinguishable by human eyes (Cornforth & Hunt, 2008). Both OxyMb (542 and 582 nm) and COMb (543 and 581nm) have twin peaks at those close wavelengths (Tang et al., 2004). They can only be distinguished due to the fact that the peak at the 580-nm region has the greatest magnitude in OxyMb, whereas the peak at the 540-nm region is the predominant one in COMb (Suman et al., 2006). DeoxyMb is purplish-red in colour. Mb has a greater affinity to CO than to oxygen, resulting in the increased stability of bright cherry-red COMb. Formation of brown MetMb results from the oxidation of the three ferrous forms to a ferric state and is associated with a decrease in the colour intensity of meat. MetMb has a water molecule at the sixth coordinate of the ferric haeme and is incapable of binding oxygen (Claus, 2001). DeoxyMb exhibits a strong absorption maximum at 557 nm, but MetMb exhibits a peak at 503 nm and the two can therefore be easily distinguished (Tang et al., 2004)

During processing of meat through methods such as smoking, drying, salting, curing, fermenting, pickling, cooking and forming, the colour of meat changes. When dark meat is

cooked, myoglobin's colour changes depending on what the meat's interior temperature is. Rare beef is cooked to 60^{o}C, and myoglobin's red colour remains unchanged. Above 60^{o}C, myoglobin loses its ability to bind oxygen, and the iron atom at the centre of its molecular structure loses an electron, converting from Fe^{2+} to Fe^{3+}. This process forms a tan-coloured compound called hemichrome, which gives medium-done meat its colour. When the interior of the meat reaches 70^{o}C, hemichrome levels rise, and the myoglobin becomes metmyoglobin, which gives welldone meat its brown-gray shade (Claus, 2001)

Several researchers who studied the variations in meat colour have attributed such variations to factors such as heat (Claus, 2001), sex (Damaziak et al., 2013), fibre composition (Listrat et al., 2016), diet (Küçükyılmaz et al., 2012) and genotype (Le Bihan-Duval et al., 1999). Research has shown that, in birds myoglobin and hemoglobin levels are lowest in the glycolytic muscles, *Pectineus* and *P. superficialis*, and highest in the oxidative *adductor* muscles and the heart and this explains the differences in the colours of these muscle types (Kranen et al., 1999). The consumption of carotenoids in green leaves and folliages has also been reported to be responsible for the yellowess of the skins and meats of birds reared outdoors (Küçükyılmaz et al., 2012).

According to Sirri et al., (2009), the sex of the bird affects the colour of poultry meat. Generally the meats of hens have been reported to be lighter than those of cocks. This they said may be attributed to differences in the metabolism of muscle fibres, which is affected by sexual hormones. In support of this view was the fact that these authors demonstrated that there were significantly higher values of yellowness (b*) and lower values of lightness (a*) in muscles of caponized male chickens compared to testosterone-producing males.

Colour measurements of meat is nowadays based on the Commission Internationale de l'Eclairage (CIE), L*a*b* scale, released in 1976. The scale is based on Opponent- Colour

Theory which assumes that the receptors in the human eyes perceive colour as the following pairs of opposites:

- L^* scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light,
- a^* scale: Red vs. green where a positive number indicates red and a negative number indicates green,
- b^* scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue (HunterLab., 2012) .

2.1.2 Texture

Texture or tenderness as applied to meat is an attribute that refers to the ease of chewing and swallowing. It is the most important attribute of meat that gives the ultimate consumer the satisfaction and joy for money well spent on the poultry meat product. Many internal and external factors contribute to the wide variation in meat tenderness. These factors can be related to the bird and the environment, processing conditions and cooking methods. Age, strain, and sex as well as environmental and nutritional stresses have all been shown to influence the variation in tenderness among meat samples (Owens et al., 2004).

The three factors that determine meat tenderness are background toughness, the toughening phase and the tenderization phase. The toughening and tenderization phases take place during the post-mortem storage period, but background toughness exists at the time of slaughter and does not change during the storage period. The background toughness of meat is defined as the resistance to shearing of the unshortened muscle and variation in the background toughness is due to the connective tissue component of muscle. While the toughening phase is similar in all carcasses under similar processing conditions, the tenderization phase is highly variable (Luciano et al., 2007).

Tenderness in poultry meat depends upon the rate and extent of the chemical and physical changes occurring in the muscle as it becomes meat. When an animal dies, blood stops circulating, and there is no new supply of oxygen or nutrients to the muscles. The muscles run out of energy, and they contract and become stiff i.e. *rigor mortis* sets in. Eventually, muscles become soft again, which means that they are tender when cooked. Anything that interferes with the formation of *rigor mortis*, or the softening process that follows it, will affect meat tenderness. For example, birds that struggle before or during slaughter cause their muscles to run out of energy quicker and *rigor mortis* forms much faster than normal. The texture of these muscles tends to be tough because energy was reduced in the live bird (De Fremery & Pool, 1960).

A similar pattern occurs due to exposure to environmental stress before slaughter (Sellier et al., 2003). High pre-slaughter stunning, high scalding temperatures, longer scalding times and machine picking can also cause poultry meat to be tough. Tenderness of portioned or boneless cuts of poultry is influenced by the time post-mortem of the deboning. Muscles that are deboned during early postmortem still have energy available for contraction. When these muscles are removed from the carcass, they contract and become tough. Aging of the meat for 6 -24 hours before deboning, may help to avoid this toughening. However, this may be costly to the processor. Early deboning, (0 to 2 hours post-mortem), causes 50 to 80 per cent of the meat to be tough. On the other hand, deboning after 6 hours may result in 70 to 80 per cent of the poultry meat being tender (Sams, 1998).

The use of post-slaughter electrical stimulation immediately after death is being practised to hasten *rigor* development of carcasses and reduce 'aging' time before deboning. This is different from energy depletion in the live bird, which causes meat to be tough. When electricity is applied to the dead bird, the treatment acts like a nerve impulse, and causes the muscle to contract, use up energy and enter *rigor mortis* at a faster rate (Bendall, 1980). In the

live bird, the same treatment causes meat to be tough but after death, the treatment causes tender deboned poultry meat within two hours post-mortem instead of the four to six hours required with normal aging. Although electrical stimulation is still in the developmental stages, it seems that processors using it can debone carcasses right out of the chiller and save on their equipment costs, time, space and energy requirements (Heinze, 2016).

2.1.3 Flavour

Flavour is another quality attribute that consumers use to determine the acceptability of poultry meat. It can be defined as a "complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting (ISO, 1992). Both taste and odour mix to contribute to the flavour of poultry meat (Figure 2.4), and it is generally difficult to distinguish between the two during consumption (Spence et al., 2010). When poultry is cooked, flavour develops from sugar and amino acid interactions, lipid and thermal oxidation and thiamin degradation (Brunton et al., 2002). These chemical changes are not unique to poultry but the lipids and fats in poultry just like in other types of meat are unique and combine with odour to account for the characteristic 'poultry' flavour. Thus it is the differences in lipid-derived volatile components between species that are mainly responsible for the species differences in flavour, whereas the precursors supplied by lean tissues generate the meaty flavour common to all cooked meats (Mottram, 1998).

Majority of the volatile compounds identified in cooked poultry meat, have been recognized in chicken (Brunton et al., 2002). However many of these have little influence on flavour of meat and relatively few make a key contribution to the odour and flavour of cooked meat (Aliani & Farmer, 2005). Melton, (1999), named sweet, sour, salty, bitter and the "umami" or savory taste as the basic tastes of meat. Hydrocarbons, aldehydes, ketones, alcohols, furans, thriphenes, pyrrols, pyridines, pyrazines, oxazols, thiazols, sulphurous compounds, and many others have been identified as the flavour and aroma compounds found

in meat (MacLeod, 1994). Few factors during production and processing affect poultry meat flavour. This means that it is not only difficult to produce a flavour defect but it is difficult to enhance flavour during production and processing. Age of the bird at slaughter affects the flavour of the meat. Chicken meat flavour also relies on several production and processing factors including the breed/strain of chicken, diet of bird, presence of free amino acids and nucleotides, irradiation, high pressure treatment, cooking, antioxidants, pH, ageing, environmental conditions, scalding etc. These ante- and post-mortem factors can influence the status of chicken meat flavour, though their effects may be too small for consumers to notice (Jayasena et al., 2018).

Heterocyclic compounds such as pyrazines, thiazoles and oxazoles are considered to be responsible for the roast flavours in foods including meat. It has been reported that a large number of heterocyclic compounds are associated with roasted, grilled, fried or pressure cooked meats, but not boiled meat, due to higher temperatures used in those cooking methods (Melton, 1999). Different alkyl pyrazines and two classes of bicyclic compounds, 6, 7-dihydro-5(H)-cyclopentapyrazines and pyrrolopyrazines, were found in meat volatiles (Mottram, 1998). It was noticed that both classes of compounds increased greatly with the increasing severity of heat treatment. However, Mottram, (1991) reported that boiled meat contained higher levels of sulphur-containing heterocyclic compounds such as thiophenes, trithiolanes, trithianes that have low odour thresholds with sulphurous, onion-like and, sometimes, meaty aromas. These compounds contribute to the overall flavour and aroma of boiled meat (Fors, 1983). The interactions that bring about flavour perceptions were summarised by Lawless as shown in Figure 2.4.

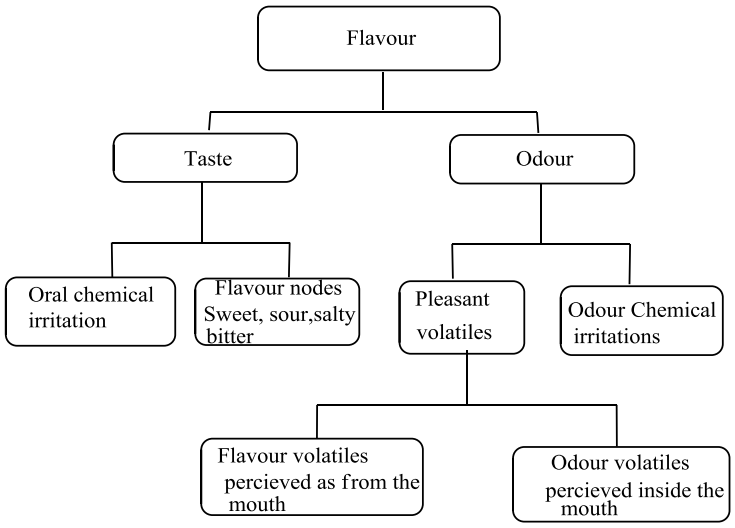


Figure 2. 4 Flavour perceptions
Adopted from Lawless, (1991)

2.2 Nutritional and chemical composition of poultry meat

Meat is defined by the Codex Alimentarius, (2005), as "all or parts of an animal that are intended for, or have been judged as safe and suitable for human consumption". Meat is composed of water, protein and amino acids, minerals, fats and fatty acids, vitamins and other bioactive components, and small quantities of carbohydrates. These are known as the chemicals of life and are very important in deciding the quality of a given meat sample. From the nutritional point of view, meat's importance is derived from its high quality protein, containing all essential amino acids and its highly bio-available minerals and vitamins. Meat is rich in vitamin B12 and iron which are not readily available in vegetarian diets (FAO, 2007). Poultry meat is ranked very high in as far as protein content is concerned (Table 2.1).

Table 2. 1 Approximate composition of Poultry Meat (g/100 g)

Component	Broiler	Turkey	Duck	Quail
Water	74.6	72.5	70.8	74.3
Ash	1.0	0.8	1.2	1.1
Protein	12.1	13.7	12.8	13.1
Lipid	11.1	11.9	13.8	11.1
Fibre	0.0	0.0	0.0	0.0
Carbohydrtes	1.2	1.1	1.4	1.4

Source: USDA (2006) as contained in Soriano, (2018)

Consumers worldwide demand a protein supply that is safe, wholesome, nutritious, abundant, and affordable. Poultry meat is supplied chiefly by chicken (*Gallus gallus*) and turkey (*Meleagris gallopavo*), although ducks, geese, guinea fowls, quails, and other fowls also contribute. Chicken is a source of protein, low in fat, which is less saturated than beef fat. Additionally, protein is a rich source of all the essential amino acids. However, eating chicken with the skin on doubles the amount of total fat and saturated fat in the dish. For this reason, chicken should best be skinned before cooking. Chicken consumption is increasing as people look for alternative ways to reduce fat such as cholesterol in their diets. To reduce fat in cooked poultry, cooking methods such as broiling, roasting, baking, simmering, or microwaving have been suggested. Chicken also provides vitamins B6 and B12, iron, zinc, and phosphorus (Soriano, 2018).

2.2.1 Proteins

The nutritional value of proteins is determined first by their content of essential amino acids and secondly by their digestibility. The amino acid composition of a protein is determined by chemical analysis and the result compared with that of a reference amino acid pattern. The score obtained from this comparison is corrected for protein digestibility. Animal foods in general are considered to be foods with high protein qualities. Red meats, poultry,

fish, eggs, milk, and milk products contain complete protein. More than 20% of these foods' energy content is protein (Young & Pellet, 1994). The human body needs 20 different amino acids, nine of which are called *essential* because the body cannot make them and must get them in the diet. Essential amino acids for adults are; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Additionally, children need arginine (Soriano, 2018).

Food proteins that supply all the essential amino acids in the proportions needed by the body are called complete. Animal foods are considered to have high protein qualities, although their qualities are not always similar because of differences in essential amino acids. The higher quality of animal protein is due to the high lysine and methionine content (Jenkins & Mitchell, 1989). A FAO–WHO, working group (1991) recommended that the amount of essential amino acids per gram of protein required by the human body should decrease with age from 434 mg in infants to 111 mg in adults (histidine excluded). Hamm, (1981) determined the differences in the amino acid profiles between broiler breast and thigh meats and concluded that, on a percent protein basis, the amounts of valine, leucine, isoleucine, and histidine were significantly greater in breast meat, and glycine, hydroxyproline, hydroxylysine, threonine, and serine were greater in thigh meat. Similarly, the results showed that the region of production and/or related management practices appear to influence the concentration of about half the amino acids. The results further showed that the levels of individual amino acids in breast muscles (related to 100% of dry matter content) ranged from 8 to 127 mg/g in pheasant chickens and from 19 to 110 mg/g in broiler chickens, while the corresponding average values in thigh muscles ranged from 14 to 132 mg/g in pheasant chickens and from 14 to 93 mg/g in broiler chickens. These results have proven the high nutritive value of pheasant meat with regard to human nutrition (Stratova et al., 2006)

The unit of skeletal muscle is the muscle fibre composed majorly of proteins that play dual pivotal roles, first as the building blocks of muscle cellular structures and, as constituent components of enzymes. Thus, they play both static and dynamic functions (Pospiech et al., 2007). Moreover, the typical traits of numerous poultry products (e.g. yield, quality, and sensory features) are dependent on the successful manipulation of protein functional properties (Smith, 2010); that can be classified into those involving the following interactions:

- Protein – water interactions including; solubility, extractability, water retention and viscosity;
- Protein – fat interactions including; fat holding and emulsification;
- Protein – protein interactions including; gelation (Smith, 2010)

Muscle proteins comprise 15-22% of the total muscle weight (about 60-88% of mass) and can be broadly divided into three groups based on their solubility characteristics as sarcoplasmic, myobrillar and stromal proteins (Xiong & Decker, 1995)

2.2.1.1 The sarcoplasmic and myofibrillar proteins

These are proteins that are intracellular and are soluble in water or in low ionic strength media. They include most of the enzymes of the glycolytic (e.g. creatine kinase, myoglobin) and oxidative (e.g. cytochromes, the flavin nucleotides, haeme pigments and the mitochondrial oxidative enzymes) pathways. The majority are globular proteins with molecular weights ranging from 17,000 (myoglobin) to 92,500 (phosphorylase b) (Tornberg, 2005). The sarcoplasmic fraction that constitutes 30 – 34% of the total protein content is soluble in water and or dilute salt solutions. This fraction covers a divergent group of proteins that controls widely differing groups of tissue functions (Pearson & Gillett, 1996).

Myofibrillar proteins constitute 50 – 55% of the total protein content and are made up of 12-14 major proteins including myosin and actin (Babij & Kee, 1994). This fraction is soluble in concentrated salt solutios. Myosin is the protein that converts chemical energy in

the form of ATP to mechanical energy, thus generating force and movement. Actin filaments, usually in association with myosin, are responsible for many types of cell movements. The binding between myosin and actin molecules occurs through cross-links between the two proteins, during the process of muscle contraction, forming the actin-myosin complex (Clark et al., 2002). The interactions of actin and myosin are also responsible for a variety of movements of non-muscle cells, including cell division; interactions that play a central role in cell biology (Cooper, 2000). Furthermore, the myofibrillar fraction includes tropomyosin, troponin, the actinins (α and β forms) and other minor regulatory proteins, which play important roles in muscles and meat (Pearson & Gillett, 1996). Myofibrillar proteins are responsible for water retention, and in muscles with low content of connective tissues, contribute significantly to meat tenderness and toughness (Zayas, 1997)

2.2.1.2 Stromal proteins (collagen, elastin)

Stroma proteins are usually measured as the insoluble proteins remaining after exhaustive extraction of all soluble muscle proteins. These proteins make up 10 – 15% of the total protein content and directly influence meat quality. For example, they; lower tenderness, decrease the emulsifying capacity, lower water holding capacity and generally decrease the nutritive value of meat (Zayas, 1997). Collagen makes up the majority of the stroma proteins and is considered the most abundant protein in the body, comprising up to one third of the total body protein (Alvarado & Owens, 2005). However, from a nutritional perspective, collagen is of lower biological value than other meat proteins. Thus, the ratio of collagen to elastin (second stroma protein) influences the biological value of meat proteins (Vognarová et al., 1996). It is found in muscle at 1- 9% of the dry, fat-free mass where it exists as a network of fibres with the ability to resist over-extension which may cause damage to the tissue. The force of contraction is transmitted to the tendons through sheets of intramuscular connective tissues which enclose the individual muscle fibres (Etherington & Sims, 1981).

Three collagenous structures can be distinguished morphologically into epimysium, perimysium and endomysium (Figure 2.5)(Etherington & Sims, 1981). The epimysium is often thick and tough and resistant to both shear and solubilization. However, it is easily (and usually) separated from cuts of meat and is generally not considered to be a factor in meat quality. The perimysium is thought to play the major role in determining meat texture differences that are related to connective tissues (McCormick, 1999). The endomysium covers individual muscle fibres and fills out spaces among them (Kurose et al., 2006). Moreover, the epi-, peri-, and endomysial sheaths determine the architecture of muscle and protect muscles from being overstretched (McCormick, 1999).

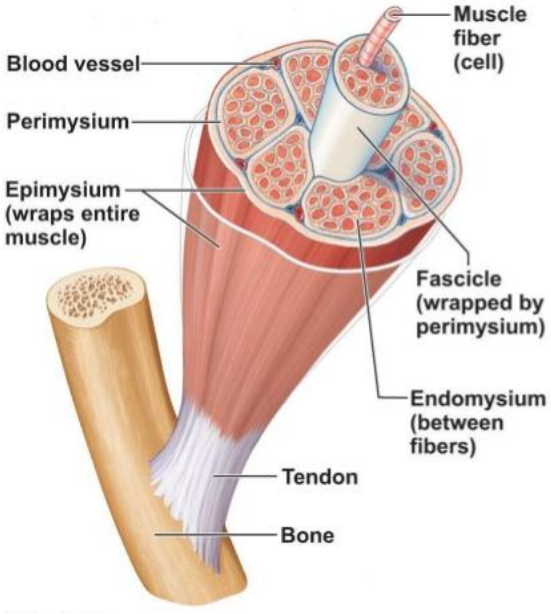


Figure 2. 5 Structure of a skeletal muscle
Source: www.medicinehack.com/2016/12/endomysium-perimysium-and-epimysium.html

So far, 26 genetically distinct collagen types have been described in vertebrates with 42 distinct polypeptide chains, more than 20 additional proteins with collagen-like domains and approximately 20 isoenzymes of various collagen-modifying enzymes. Based on structure and organization collagens can be grouped into fibril-forming collagens, fibril-associated collagens (FACIT), network-forming collagens, anchoring fibrils, trans-membrane collagens, basement membrane collagens and others with unique functions (Ku¨hn, 1986). The most

abundant and widespread family of collagens with about 90% of the total collagen is represented by the fibril-forming collagens. Types I and V collagen fibrils contribute to the structural backbone of bone and types II and XI collagens predominantly contribute to the fibrillar matrix of articular cartilage (Gelse et al., 2003). Their torsional stability and tensile strength lead to the stability and integrity of these tissues. Type IV collagens with a more flexible triple helix assemble into meshworks restricted to basement membranes. Microfibrillar type VI collagen is highly disulfide cross-linked and contributes to a network of beaded filaments interwoven with other collagen fibrils (van der Rest, 1991).

Fibril-associated collagens with interrupted triplehelices (FACIT) such as types IX, XII, and XIV collagens associate as single molecules with large collagen fibrils and presumably play a role in regulating the diameter of collagen fibrils (Mayne, 1989). The basic structural unit of collagen is tropocollagen, 300 Å long, 14 Å thick and has a molecular weight of about 300,000. It consists of three polypeptide chains, each with over 1000 amino acid residues. The most prominent amino acid is glycine, accounting for about one-third of all amino acids in collagen. Proline and hydroxyproline (22%) and alanine (11%) are the next most abundant amino acids. Collagen also contains another amino acid, hydroxylysine, which rarely, if ever, occurs in other proteins. The typical amino acid sequence in a collagen polypeptide chain is Gly-X-Y, where proline (~20%), may occupy positions X or Y, but hydroxyproline may occupy position Y only. Glycine is required at every third position and is tightly packed at the centre of the triple helical structure. The post-translational 4-hydroxylation of most prolines in the Y position is required for triple helical stability (Bachinge et al., 2010).

The secondary structure of tropocollagen is helical on two levels: each polypeptide chain has a left-handed helical sense, with 3.3 amino acids per turn and a total linear distance of 9.6 Å per turn (Bhattacharjee & Bansal, 2005). This gives a pitch of 9.6/3.3- 2.91 Å for

each amino acid. Three such helical polypeptide chains are assembled into a tropocollagen cable consisting of three intertwined polypeptide chains, forming a superhelix with right-handed sense (Anne et al., 1999). The superhelix, and each subhelical component, has a total of about 29 turns (104 Å per turn). Each turn consists of 36 amino acids, giving a total of about 1044 amino acids per strand and a total of about 3100 amino acids per tropocollagen molecule. Moreover, the subhelices are arranged in the superhelix in such way that the glycine residues are on the interior of the superhelix and in contact with each other. The X and Y residues face the environment and are therefore able to accommodate any bulky side chains (Bezkorovainy & Rafelson, 1995).

Collagen fibrils and the fibrous matrices they form are stabilized by covalent crosslinks formed by spontaneous reaction of an allysine or hydroxyallysine with an unmodified lysine or hydroxylysine residue on an adjacent polypeptide chain. The initial crosslinks formed are dysfunctional and are usually described as reducible crosslinks because they possess Schiff base double bonds. With maturation divalent crosslinks disappear from many tissues and may be replaced by mature, non-reducible crosslinks (McCormick & Thomas, 1998). It has been reported that the progression of crosslinking occurs significantly faster in avian skeletal muscle than in other mammals (McCormick, 1999).

The content of collagen has been the ultimate goals in numerous studies. It was observed that collagen content of dark coloured muscles (red meat) was higher than in light coloured muscles (white meat). Moreover, breast muscles of broiler chickens were found to be characterized by the highest collagen content at the ages of two weeks and five weeks, respectively. Kerr et al., (2001) reported that the collagen of fast growing animals is less mature than that of slow growing animals at the same slaughter weight.

Elastin is the extracellular matrix protein responsible for the resilience of tissues such as skin, arteries and lung. It is an insoluble, hydrophobic and extensively cross-linked protein

forming fibres which are present in variable amounts depending on the tissue; and with functions restricted to elasticity (Debelle & Alix, 1999). It has a molecular weight of about 72,000 and contains 800-850 amino acid residues (Bezkorovainy & Rafelson, 1995).

2.2.2 Amino acids

Amino acids (AAs) are organic compounds with both $–NH_2$ (amino) & –COOH (carboxyl) functional groups. There are about 20 amino acids in a protein hydrolysate and with a few exceptions; all have the general structure shown in Figure 2.6.

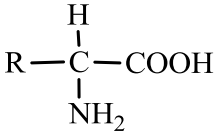


Figure 2. 6 The general structure of amino acids
Adopted from Grosch, (2014)

In the simplest case, aminoacetic acid (glycine); R = H. In other amino acids, R is an aliphatic, aromatic or heterocyclic residue and may incorporate other functional groups; and all amino acids except glycine exhibit optical activity occurring as the L- or D-isomers. These isomeric configurations are defined with reference to the structure of glyceraldehydes. Except for proline, all protein amino acids have a primary amino group and a carboxyl group linked to the α-carbon atom. In a β-AA, an amino group links to the β-carbon atom (Wu, 2009). A series of amino acids joined by peptide bonds forms a polypeptide chain, and each amino acid unit in a polypeptide is called a residue (Liu & Liu, 2016). There are a number of ways of classifiying amino acids. One of which is based on their side chains which are the deciding factors for intra- and intermolecular interactions in proteins. Based on this, amino acids can be classified as:

- Amino acids with nonpolar, uncharged side chains: e. g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan and methionine. These exhibit limited

solubility in water.

- Amino acids with uncharged, polar side chains: e. g., serine, threonine, cysteine, tyrosine, asparagine and glutamine. These are fairly soluble in water due to the polarity of the side chains.
- Amino acids with charged side chains: e. g., aspartic acid, glutamic acid, histidine, lysine and arginine. These are highly soluble in water due to the charges they carry.

Amino acids can be degraded into carbon skeleton and ammonia and these can form another basis of their categorisation. Glucogenic amino acids (including Ala, Cys, Gly, Ser, Thr, Trp, Asn, Asp, Phe, Ile, Met, Val, Arg, Glu, Gln, His, and Pro) are glucose precursors and can be degraded to pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate. On the other hand ketogenic amino acids (Leu and Lys) can be converted to fatty acids or ketone bodies and degraded to acetyl-CoA or acetoacetate (Fei, 2004).

Amino acids can also be categorised based on their source to the animals. In poultry, 22 amino acids are needed to form body proteins, some of which can be synthesized by the bird (non-essential amino acids: Cys, Asp, Ser, Glu, Pro, Gly, Ala, Tyr, His, Arg), whereas others can not be made at all or in sufficient quantities to meet metabolic needs (essential amino acids, EAAs: Thr, Val, Met, Leu, Ile, Phe, Lys) (Applegate, 2014). Proteins with a high content of essential amino acids are the most important components of poultry meat. Essential amino acids must be supplied by the diet, and a sufficient amount of non-essential amino acids must also be supplied to prevent the conversion of essential amino acids into non-essential amino acids (Applegate, 2014). In addition, the type of EAAs in food varies, causing a variation in digestion quality and, ultimately, differences in protein value among food sources (Aronal et al., 2012).

It has been reported that increasing the lysine concentration in the feeds of broilers and slow growing layers leads to greater proportions of lysine in the total body protein, although a

greater effect is observed in broilers (Vieira & Angel, 2012). Moreover, research has found that increasing the level of lysine in the diet of broilers (beyond the requirements for growth) reduces drip loss of breast muscles during storage by increasing its ultimate pH (Berri et al., 2008). However, reports also indicate that dietary lysine inadequacy reduces breast meat yield compared with other muscles hence, according to Nasr & Kheiri, (2012), it is very important to define dietary AA needs for optimal growth and meat yields in poultry.

Another amino acid of great importance is methionine, which together with lysine, are two essential precursors of L-carnitine that plays important roles in lipid and energy metabolism in poultry (Bouyeh, 2013). Methionine is one of the sulfur-containing amino acids and has a strong effect on the activity of glutathione reductase and glutathione transferase (GSH-Px) (Blaszczyk et al, 2010). It has been reported that broilers in general have a high methionine requirement that cannot be supplied by the corn and soybean fraction of diets; therefore, birds require an additional ingredient source of methionine (Moritz et al., 2005). The amino acid pattern of body protein may be affected by numerous factors, including genotype and nutrition i.e. the crude content of the diet.The ratios of amino acids in muscle and other tissues in the body of the bird are constant. Birds consuming lower protein levels synthesize smaller amounts of protein and so need less of each amino acid, and vice versa (Fatufe et al., 2004; Pesti, 2009).

2.2.3 Lipids

Lipids are naturally occurring molecules from plants or animals that are soluble in nonpolar organic solvents such as chloroform, benzene, acetone etc. Lipid molecules contain large hydrocarbon portions and not many polar functional groups, which accounts for their solubility behaviour. Lipids have a variety of biological roles; they serve as fuel molecules, highly concentrated energy stores, signal molecules, and components of membranes (Berg et al., 2002a). Lipid compounds may be categorized based on their chemical structures and

biological functions. One such classification is presented in Figure 2.7. Of particular interest as far as health and poultry meat quality is concerned are the simple lipids; in particular oils and fats whose proportions in the meat play a big role in determining the nutritional quality, the stability and shelf life of the poultry meat.

Oils and fats (the triglycerides) are the most abundant of all lipids. They constitute about 98% of total dietary lipids; the remaining 2% consisting of phospholipids and cholesterol and its esters. They are the major components of storage or depot fats in plant and animal cells but are not normally found in membranes. They are nonpolar, hydrophobic molecules since they contain no electrically charged or highly polar functional groups.

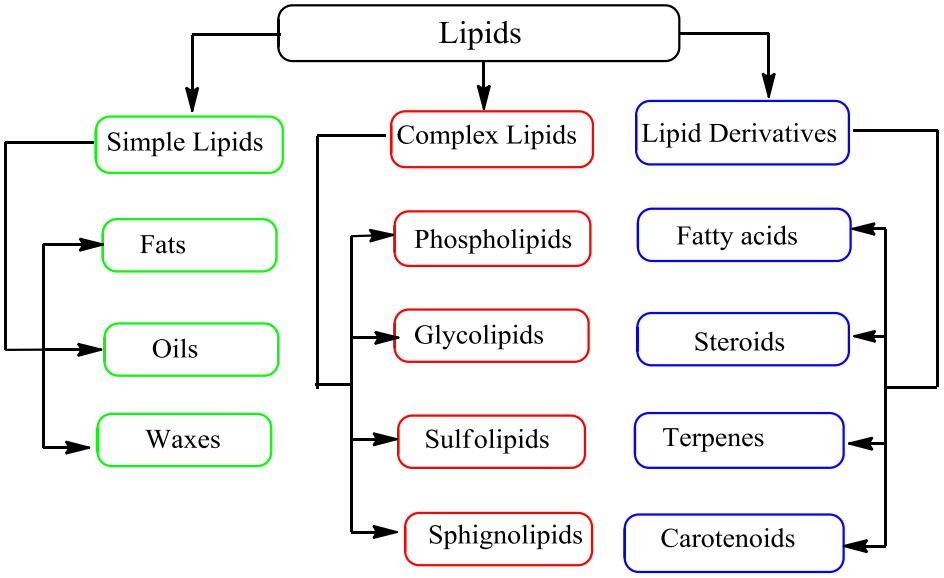


Figure 2. 7 Classification of lipids
Adopted from Dąbrowska et al., (2015).

Oils and fats differ in that oil is a mixture of triglycerols that is liquid at room temperature because it contains a high proportion of unsaturated fatty acids while fat is a mixture of triglycerols that is solid at room temperature because it contains a high proportion of saturated fatty acids (Endo, 2018). Wheras the body can store the carbohydrate glycogen in meagre amounts, sufficient to supply energy need of a day only; triglycerides can be stored in quantities, sufficient to supply the energy needs of the body for many months, as in the case

of obese persons. Triglycerides are therefore much better adapted than glycogen to serve as storage form of energy. Further they are not only stored in large amounts but also yield over twice, as much energy as carbohydrates. Since fats tend to remain in the stomach longer than carbohydrates and are digested more slowly, they also have greater satiety value than carbohydrates. Fats help the body in absorbing the fat soluble vitamins, A, D, E and K. In a normal man, weighing 70 kg, at least 10-20% of the body weight is lipid, the bulk of which is triacylglycerol (TAG). TAG is found in all organs of the human body, particularly in adipose tissues (Endo, 2018).

Chemically, triglycerides are esters of glycerol with 3 fatty acid molecules. When assymetrical, two optical isomers may occur as shown in Figure 2.8 .The naturally occurring fats are of L-type.

Figure 2. 8 Two optical isomers of triglycerides
Adopted from Chuan; (2008).

Based on the lipid content of the muscles of the various livestock species, chickens are recognized as efficient sources of lean meat. However, the long-term intense selection for increased juvenile growth in broiler chickens has led to increased fat deposition in the chicken abdomen (Guo et al., 2011). This is an unfavorable trait for producers and consumers because excessive fat is considered a wasted dietary energy and a waste product with low economic value, which also reduces the carcass yield and affects consumer acceptance. Research has shown that modern broiler strains contain 15% to 20% fat and >85% of this fat is not physiologically required for body functions (Fouad & El-Senousey, 2014). However, adipose tissue in meat is desirable, to some extent, to give a finished appearance to a carcass (Musa et

al., 2007). In avian species, the amount of fat that accumulates in the body depends on the available plasma lipid substrate, which originates from the diet or *de novo* lipogenesis in the liver (Fouad & El-Senousey, 2014). The biological mechanisms that regulate the synthesis and degradation of lipids and lipid transport in plasma are of great significance to animal in zootechnical production (Musa et al., 2007).

The important properties of oils and fats in as far as health and poultry meat quality are concerned are derived from their precursors, the derived lipids. The group derived lipids is a "catch all" group in Bloor's classification. It includes the hydrolysis products of simple and compound lipids and also various other compounds such as steroids, terpenes fatty acids, alcohols, fatty aldehydes, ketones etc. However, the most important derived lipids impacting on poultry meat quality and on health of the consumers are the fatty acids.

2.2.3.1 Fatty acids

Among the most biologically significant properties of lipids is their hydrophobic nature. These properties are mainly due to a particular component of lipids; fatty acids (Berg, et al., 2002b). Fatty acids, occurring both as free acids and as parts of complex lipids, play key roles in metabolism (storage and transport of energy), as essential components of all membranes, and as gene regulators. They are also important for thermal and electrical insulation, and for mechanical protection (Rustan & Drevon, 2005). Fatty acids are divided into two categories based on structural and chemical properties as: (1) saturated and (2) unsaturated. Saturated fatty acids (SFAs) do not contain any pie bonds along the hydrocarbon chain. Unsaturated fatty acids contain at least a pie bond along the hydrocarbon chain enabling the addition of more hydrogen or other atoms at that/those points.

The presence of the double bonds, which naturally occur in the *cis* configuration, puts a rigid 30° bend in the hydrocarbon chain of unsaturated fatty acids that interferes with packing in space. This causes reduced van der Waals interactions which causes melting points

to decrease making the lipids to be fluidy like in vegetable oils. These lipids do not clog our arteries. When solid, they tend to be more flexible and this makes them very suitable for inclusion into cellular and structural membranes. Saturated fatty acids (SFAs) are able to pack more closely and this increases the van der Waal's force between them leading to higher melting and boiling points, as seen in many fats.

Unsaturated fatty acids can be further categorised based on the extent of unsaturation into monounsaturated fatty acids (MUFAs) which contain only one double bond and polyunsaturated fatty acids (PUFAs), which contain more than one double bonds (Panickar & Bhathena, 2010). As already explaind above, the extent of unsaturation should determine the boiling and melting points of the fatty acid. PUFAs generally have lower melting and boiling points compared to their MUFA and SFA analogues of comparable relative molecular masses. Melting points of fatty acids also increase with chain length (Berg et al., 2002a) but decrease with the extent of branching. They are generally higher in PUFAs with *trans* as opposed to the *cis* configuration. In the 18C fatty acid series, stearic acid (18:0) melts at 69.6^{o}C, oleic acid (18:1) at 13.4^{o}C, linoleic (18:2) at -9^{o}C and α-linolenic (18:3) at -17^{o}C. Variation in fatty acid composition greatly affects the firmness or softness of the fat in meat (Wood et al., 2003).

In PUFAs the first double bond may be found between the third and the fourth carbon atom from the terminal (omega) carbon; giving rise to omega-3 (ω-3 or n-3) fatty acids. If the first double bond is between the sixth and seventh carbon atom, then the molecules are called omega-6 (ω-6 or n-6) fatty acids. The double bonds in PUFAs are normally conjugated and can be introduced at any position along the chain except the n-3 and n-6 that already contain one (Panickar & Bhathena, 2010). Two PUFAs of utmost significance are linoleic acid [18:2 (ω-6 omega)] and α-linolenic acid [18:3 (ω-3 omega)]. These two, referred to as essential fatty acids (EFAs) are sources for the production of important longer chain PUFAs such as prostaglandins, thromboxanes and leukotrienes; dynamic but short lived compounds which

are involved in intracellular communication and control of blood vessels and other body functions (Gogus & Smith, 2010).

There are two essential fatty acids (EFAs) in human nutrition: alphalinolenic acid (ALA), an omega-3 fatty acid, and linoleic acid (LA), an omega-6 fatty acid. Humans must obtain EFAs from foods because the human body cannot make them. EFAs are required for the structure of cell membranes and, because they are unsaturated, they help keep membranes flexible. They are precursors of long-chain fatty acids, some of which are converted to powerful compounds that affect many biological processes, including inflammation and cell signaling. EFAs affect gene expression – that is, they turn genes on for the making of cell proteins (Sampath & Ntambi, 2004). EFAs also have antibacterial actions and are found in breast milk (Das, 2006). Indeed, breast milk is rich in LA and contains more of ALA than any other omega-3 fatty acid (Bopp et al., 2005).

ALA and LA are required in our diet because our bodies cannot make them. For this reason, ALA and LA are essential nutrients just like calcium, potassium, vitamin C and folate. The long-chain fatty acids derived from ALA and LA are not essential because the body can make them from ALA and LA. However, because of the crucial role that they play in maintaining health and preventing diseases, the long-chain fatty acids in the omega-3 family and many in the omega-6 family are also called “essential” in the medical sense (Qiu, 2003).

2.2.3.2 Metabolism of alpha-linolenic acid (ALA)

It is reported that about 96% of dietary ALA appears to be absorbed in the gut (Burdge, 2006). After absorption, ALA has several metabolic fates: 1) It can undergo β-oxidation to produce energy; 2) It can be recycled to make other fatty acids; 3) It can serve as a substrate for ketogenesis, the process of making ketone bodies, which are very good for brain health at old age; 4) It can be stored in adipose tissues for later use; 5) It can be incorporated into the phospholipids of cell membranes, where it affects membrane activities;

and 6) It can be converted to long-chain omega-3 fatty acids like eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), which have important functions in many types of cells and organs (Figure 2.9).

2.2.3.3 Metabolism of linoleic fatty acids (LA)

Like the metabolism of ALA, LA is converted to long-chain omega-6 fatty acids by a series of desaturations and elongations (Figure 2.9). Three fatty acids in the ω-6 pathway are worth noting. Gamma-linolenic acid (GLA) is separate and distinct from ALA, the essential omega-3 fatty acid, and the two fatty acids should not be confused. GLA is converted to dihomo-gamma-linolenic acid (DGLA), a precursor of certain eicosanoids that tend to be somewhat mild in their biologic effects. GLA is found in evening primrose, borage and black currant oils. Arachidonic acid (AA) is the precursor of powerful eicosanoids, several of which promote the clumping (aggregation) of blood platelets, the clotting of blood within blood vessels (thrombosis) and inflammatory reactions (Danijela et al., 2013).

Site of activity	ω -6 Fatty acids	Enzymes	ω-3 Fatty acids
	Linoleic acid 18:2		α- Linoleic acid 18:3
	⇩	Δ^6 desaturase	⇩
	γ-Linoleic acid 18:3		Octadecatetranoic acid 18:4
	⇩	elongase	⇩
	Dihomo-γ-linoleic acid 20:3		Eicosatetraenoic acid 18:4
	⇩	Δ^5 desaturase	⇩
Endoplasmic reticulum	Arachidonic acid 20:4		Eicosapentaenoic acid 20:5
	⇩	elongase	⇩
	Adrenic acid 22:4		Docosapentaenoic acid
	⇩	elongase	⇩
	Tetracosatetraenoic acid 24:4		Tetracosapentaenoic acid 24:5
	⇩	Δ^6 desaturase	⇩
	Tetracosapentaenoic acid 24:5		Tetracosahexaenoic acid 24:6
	⇩	β−oxidation	⇩
Peroxisome	Docosapentaenoic acid 22:5		Docosahexenoic acid 22:6

Figure 2. 9 Metabolism of essential fatty acids
Adopted from Rustan & Drevon, (2005).

AA is the most tightly regulated fatty acid in cell membrane phospholipids because it affects the way cells behave, and its actions have far-ranging effects (Seeds & Bass, 1999). Diets high in LA or AA may result in an over-abundance of AA-derived eicosanoids that, in turn, may result in an over-active immune system that contributes to chronic diseases like coronary heart disease, diabetes and cancer (Calder, 2006; Levick et al., 2007). Docosapentaenoic acid (DPA) with the formula 22:5n-6 is a member of the omega-6 family. It should not be confused with the omega-3 DPA (22:5n-3). The metabolisms of the two EFAs are summarised in Figure 2.9 (Rustan & Drevon, 2005).

It is worth noting that mammals cannot interconvert the ω-3 and ω-6 fatty acids – they are two separate, distinct families. Furthermore, their metabolism requires the same enzymes, resulting in competition between the two families (Burdge, 2006). An excess of one family of fatty acids can interfere with the metabolism of the other, reducing its incorporation into tissue lipids and altering its biological effects (Burdge & Calder, 2005).

2.2.3.4 Dietary fatty acid ratios

The dietary ω-6/ω-3 ratio affects inflammation and gene expression, thus influencing the development of chronic disease. The ω-6/ω-3 ratio may be as high as 17:1 in some Western diets (Simopoulos, 2006). It is roughly 10:1 in the U.S. diet (Kris-Etherton et al., 2002). Some western diets however contain ratios as high as 30:1 or even 50:1 which results from very high levels of ω-6 PUFAs and relatively low consumption of ω-3 PUFAs (Zduńczyk & Jankowski, 2013). The ω-6 to ω-3 ratio of diets during human evolution was estimated to be close to 1:1 (Vahmani et al., 2015). People with high intakes of meat, French fries, some fast-food products and foods fried in omega-6-rich vegetable oils will have a higher ω-6/ω-3 ratio than average. The ω-6/ω-3 ratio recommended by international agencies and some European countries ranges from 4:1 to 10:1 (Gebauer et al., 2006). The U.S.

Institute of Medicine (IOM) supports a ratio of 5:1 for the U.S. and Canadian populations (National Institute of Medicine, 2002).

Numerous studies have shown that in secondary prevention of cardiovascular diseases, a ratio of 4:1 was associated with a 70% decrease in total mortality. A lower ω-6/ω-3 PUFA ratio decreased the risk of breast cancer in women and suppressed inflammations in patients with rheumatoid arthritis. The ratio of 2.5:1 reduced rectal cell proliferation in patients with colorectal cancer (Zduńczyk & Jankowski, 2013).

One consequence of the dietary imbalance between ω-6 and ω-3 fats is a high ratio of ω-6 to ω-3 fatty acids in cell membranes. An imbalance in the ω-6/ω-3 ratio in tissues and blood can have adverse effects, including the overproduction of pro-inflammatory eicosanoids, many of which are derived from arachidonic acid, an ω-6 fatty acid. Excess eicosanoids, in turn, stimulate the release of inflammatory cytokines and acute-phase proteins. The end result is low-grade chronic inflammation that contributes to health problems such as atherosclerosis, blood viscosity, vasospasm, vasoconstriction as well as reduced bleeding time (Vahmani et al., 2015), Alzheimer disease, cancer, cardiovascular disease, metabolic syndrome, obesity, osteoporosis, type 2 diabetes and periodontitis (Calder, 2006).

Poultry meat has been considered as one of the main sources of PUFA for human diets, in particular ω-3 PUFA (Ponte et al., 2008). Moreover, it has been shown that the ω-3 fatty acid content of poultry meat particularly ALA, can be readily improved through dietary supplementation with fish oil by-products (Ponte et al., 2008). Fish oil is the most common long-chain ω-3 PUFA supplement used but is unsustainable and reduces the oxidative stability of the meat. This marine supplement represents a rich source of PUFAs and especially the more highly unsaturated fatty acids such as DHA and EPA. Mirghelenj et al., (2009), reported that the DHA content in breast and thigh meat of birds fed diet with 0-2% fish oil increased from 0.046 and 0.086 mg/g to 0.166 and 0.27 mg/g, respectively.

However, whereas the inclusion of fish oils in feed formulations for birds improved the nutritional value of the products, there was some decrease in sensory quality reported as fishy off-flavours. This reduction in sensory quality, according to Mooney et al., (1998), could be alleviated by using marine algae. They reported that although the overall acceptability of the meat from broilers fed marine algae was reduced (compared with the control), it was more acceptable than meat from birds fed fish oil, suggesting that the oxidative stability of the meat from birds fed algae rather than fish oil was greater. The effect of vegetable oils in improving broiler meat quality cannot be overlooked (Zduńczyk & Jankowski, 2013). A study by Kamran Azad et al., (2009), reported that the inclusion of full-fat flaxseed and canola seed significantly increased the concentration of omega-3 fatty acids and decreased the content of arachidonic acid and ω-6: ω-3 PUFA ratio in the meat.

Increasing MUFA and PUFA i.e. a high PUFA/SFA contents in poultry meat can be beneficial for human health (Mašek et al., 2013). Saturated fatty acids in meat as a source of fat in the human diet are associated with several diseases of modern civilization such as obesity or cancer (Zymon et al., 2007). Additionally, dietary SFAs cause an increase in serum total and LDL cholesterol and therefore increase the risk of heart disease (Tartrakoon et al., 2016). There is convincing evidence that: replacing SFAs (C12:0 – C16:0) with PUFAs decreases LDL cholesterol concentration and the total:HDL cholesterol ratio. A similar but lesser effect is achieved by replacing these SFA with MUFAs (FAO/WHO, 2008). Research has shown that the ratio of PUFA to SFA (P/S) has great nutritional implications and it is taken as a measure of the propensity of the diet to influence the incidence of coronary heart disease; the recommended ratio should be in the range of 0.4 - 0.7 (Wood et al., 2003). Since some meats naturally have a P:S ratio of around 0.1, meat has been implicated in causing the imbalanced fatty acid intake of today's consumers. For this reason, ways to improve the P: S ratio during meat production are required. From this point of view poultry meat is

characterized by a good P:S ratio (> 0.60) due to the higher incidence of PUFA fractions. Compared with muscle of the other species, poultry breast muscle have higher C18:2 and C20:4 and lower C18:0, which are largely responsible for the higher PUFA/SFA ratios observed for other species (Rule et al., 2002).

2.2.4 Cholesterol

Cholesterol (from the Ancient Greek *chole-* (bile) and *stereos* (solid), followed by the chemical suffix *-ol* for an alcohol) is an organic molecule. It is a sterol (or modified steroid), a type of lipid molecule, and is biosynthesized by all animal cells, because it is an essential structural component of all animal cell membranes. François Poulletier de la Salle first identified cholesterol in solid form in gallstones in 1769. However, it was not until 1815 that chemist Michel Eugène Chevreul named the compound "cholesterine (Olson, 1998). Cholesterol is an unsaturated alcohol of formula $C_{27}H_{45}OH$ that is a waxy substance made by animal liver and also supplied in the diet through animal products such as beef, poultry, fish and dairy products. Cholesterol is needed in the body to insulate nerves, make cell membranes and produce certain hormones, and it is an important lipid in some membranes (Ma, 2004).

The molecular structure of cholesterol (Figure 2.10) includes a tetracyclic fused ring skeleton, with a single hydroxyl group at carbon 3, a double bond between carbons 5 and 6, and an iso-octyl hydrocarbon side chain at carbon 17. The hydroxyl group in cholesterol is very important, because it gives the otherwise hydrophobic compound its amphiphilic character and therefore orients the molecule in membranes. Further, the hydroxyl group can also mediate the hydrogen bonding of cholesterol with water and possibly with other lipid components of cellular membranes (Ohvo-Rekilä et al., 2002).

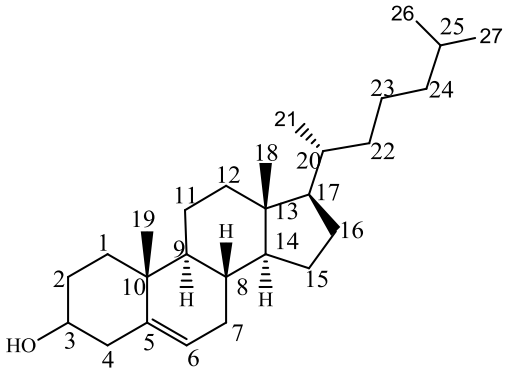


Figure 2. 10 The chemical structure of cholesterol
Adapted from: https://pubchem.ncbi.nlm.nih.gov/compound/5997

Poultry meat is characterized by a low cholesterol content (broiler Pectoralis muscle, 47.41 mg/100 g muscle (Chizzolini et al., 1999); making it healthier than other meat products i.e. beef 66 mg/100 g, pork 65 mg/100 and lamb 50 mg/100 (Chizzolini et al., 1999). However, other studies on broiler chicken reported higher cholesterol values; for example, Salma et al., (2007) reported an average cholesterol content of 93.6 mg/100g of meat in Pectoralis major of 56 day old male Chunky broilers; while, (Maiorano et al., 2012) reported cholesterol values ranging from 70.45 to 78.12 mg/100g in 42 day old broiler chickens. However, a recent study by (Tavaniello et al., 2018) reported cholesterol levels ranging from 46.74 to 49.57 mg/100 g. Their findings were similar to those reported by Pilarski et al., (2005), (ranging from 49.3 to 54.7 mg/100 g) in breast muscles of 42-d-old broiler chickens but were lower (- 50%) than that reported by Maiorano et al., (2012).

The discrepancies in these reports could be explained by the use of different analytical methodologies for cholesterol quantification and sampling (Bragagnolo & Rodriguez-Amaya, 2002), diet and breed (de Oliveira et al., 2016), age, and sex (Wang et al., 2015). Further, the differences in muscle cholesterol content among livestock species are explained by variations in absorption and biosynthesis of cholesterol, lipoprotein metabolism, diet, muscle fibre type distribution, genetic variation, subcutaneous and intramuscular fat, body weight and cell size (Dinh et al., 2011). In poultry meat, the cholesterol content is affected by the type of retail cut,

(dark or white meat) and the presence or absence of skin in the cut. Poultry skin has the highest cholesterol concentration compared with poultry meat or fat. The difference in cholesterol content between white and dark poultry meat is more pronounced than that between white (predominantly glycolytic) and red (predominantly oxidative) muscles in beef and pork. In general, raw poultry meat has approximately 27 to 90 mg cholesterol/100 g and cooked poultry meat contains around 59 to 154 mg/100 g (Chizzolini et al., 1999).

2.2.5 Vitamins and mineral elements

Micronutrients, as opposed to macronutrients (proteins, carbohydrates and fats), are comprised of vitamins and minerals which are required in small quantities to ensure normal metabolism, growth and physical well-being. Micronutrients differ from macronutrients in key characteristics. Whearas macronutrients are consumed in large amounts, micronutrients are ingested in much smaller amounts (mg to μg per day). Macronutrients provide sources of energy, needed to fuel the body, maintain cellular hydration and provide the body structures to perform work. Micronutrients on the other hand enable the use of macronutrients for all physiologic processes and despite their relatively low amounts in the diet and the body, vitamins and minerals are key regulators of health (Lukaski, 2004). Micronutrient deficiencies are very common in many of the developing countries where they lead to major adverse health consequences such as impairments in growth, immune function, mental and physical development and reproductive outcomes that cannot always be reversed by nutrition interventions (van Huis et al., 2013).

Chicken meat is a very nutritious food as it contains a variety of vitamins and minerals, and with a couple of exceptions, these micrronutrients are found fairly equally in both white and dark meat. Chicken wings, legs and thighs are richer in zinc and vitamin B12, and legs and thighs contain more of the B vitamin biotin. Chicken meat is a source of all of the B complex vitamins; thiamin (B1), riboflavin (B2), niacin (B3), pantothenate (B5),

pyridoxine (B6), folate (Kelly & Doug, 2014). While whole grains are rightfully promoted as good sources of B vitamins, foods like chicken are an important source as well. Chicken legs and thighs, both skinless and with skins on, as well as chicken wings are very high in vitamin B12. Apart from the B complex vitamins, poultry meat is also a good source of the lipophilic vitamins A, E and K and the water soluble vitamin C (Soriano-Santos, 2010).

Reports have shown that rearing systems and diet have influences on the micronutrient composition of poultry meat. For example as George Mateljan Foundation (2018) reports, pasture raised chickens are very rich sources of both vitamins and minerals; and the levels of these micronutrients determined in 113.4 g cuts of breast muscles are shown in Table 2.2.

Table 2. 2 Micronutrient contents of roasted breast of chicken pasture raised

Vitamin	Amount	Mineral	Amount
B1	0.08 mg	Calcium	17.01 mg
B2	0.13 mg	Copper	0.06 mg
B3	15.55 mg	Iron	1.18 mg
B6	0.68 mg	Magnesium	32.89 mg
B12	0.39 mg	Manganese	0.02 mg
Choline	96.73 mg	Phosphorus	258.55 mg
Folate	4.54 mg	Potassium	290.30 mg
Pantothenic acid	1.09 mg	Selenium	31.30 mg
Vitamin C	0.00 mg	Sodium	83.91 mg
Vitamin A	23.81 IU	Zinc	1.13 mg
Vitamin D	5.67 IU		
Vitamin E	0.31 ATE* 0.46 IU		
Vitamin K	0.34 μg		

* Alpha tocopherol equivalent
Source: www.whfoods.com/genpage.php?tname=foodspice&dbid=116

As reported by Kłoczko, (2004) the content of the compounds in the skeletal muscles of birds is about 1-1.5%. Poultry is rich in both the fat soluble vitamins A, D, E and K that have no direct roles in energy metabolism; but play supportive roles for energy use. β-

carotene; a precursor of vitamins A and E acts as an antioxidant in reducing muscle damage and enhancing recovery from physical activity (Lukaski, 2004). Vitamin K acts as a cofactor for the carboxylation of certain glutamic acid residues in specific vitamin K-dependent proteins, to form γ-carboxyglutamic acid (Gla) (Elder et al., 2006). The B vitamins and vitamin C are water soluble. Vitamin C plays a role as an antioxidant while the B vitamins help to regulate energy metabolism by modulating the synthesis and degradation of carbohydrates, fats, proteins and bioactive compounds. Vitamin B12 is required for haemoglobin synthesis (Lukaski, 2004).

Mineral elements in meat are classified as either essential or nonessential, depending on whether or not they are required for human nutrition and have metabolic roles in the body. Nonessential elements may further be categorized as either toxic or nontoxic (Soriano-Santos, 2010). Muscle tissue is an important source of phosphorus and potassium, but is low in calcium and is a moderate source of magnesium. The levels of sodium in raw meats are not significant but may increase during processing when it may be added as part of curing and preserving or as a flavour-enhancing ingredient.

Poultry meat; in particular the liver is an important source of essential microminerals such as iron, zinc, copper, manganese, selenium, cobalt, iodine and molybdenum due to their exclusively higher bioavailability than from plant sources. Iron plays a key role in oxygen transport and accumulation through the haemoglobin and myoglobin respectively. This mineral is present in meat and meat products as non-haem iron (nheFe) and haem iron (heFe) being the most efficiently absorbed form from the diet (Lombardi-boccia & Beatriz, 2002). Selenium is an antioxidant but also influences the production of feathers and maintenance of cellular integrity in avain species. It is mainly added as a supplement in poultry feeds in the form of yeast derived selenium to which animals and poultry have become adapted. Whereas organic selenium accumulates in tissues such as the liver, brain, and muscles, its

concentration is highest in the bird's feathers (Perić et al., 2009). White meats, such as breast meat of broilers, are reportedly lower in Fe content than red meats, such as beef (Seo et al., 2008). Reports also indicate that Se-rich meat is juicier, crispier, and more appealing to sight. Many farmers therefore use Se in combination with other antioxidants such as the tocopherols (vitamin E) for fodder enrichment (Suchý et al., 2014). Zinc has been reported to always exhibit interactions with other elements such as iron, copper, manganese, cobalt and molybdenum. It has also been applied as a growth stimulator of farm animals for several decades, and is important as an activator of significant enzymes and hormones (Herzig et al., 2009). A study by Karamouz et al., (2011) has shown that Zn acts as an antioxidant which reduces the cell membrane damage due to free radicals, which in and in doing so, changes the immunological status of the animal.

In western societies, where the consumption of meat is high, more than 70% of zinc is provided by animal products, especially meat. A diet of mixed dark and white chicken meat is reported to contain 8.5 – 9.0 mg/kg of zinc in the edible portion and 100 g povides, roughly 6% of the daily value. Whereas seeds, nuts and wholegrain cereals also contain high levels, the bioavailability of zinc in these foods can be reduced by their content of appreciable levels of phytate (Soriano-Santos, 2010).

Chapter 3

INDICATORS OF POULTRY MEAT QUALITY DETERIORATION

3.0 Introduction

From the time of slaughter to consumption, poultry meat undergoes several physico-chemical and or biological transformations that may be indicators of deterioration. These processes have a direct bearing on the quality, shelf life and acceptance of poultry meat. They determine how long poultry meat shall maintain its attractiveness and saleable interest and value. The processes include but are not limited to the following.

3.1 pH of poultry meat

pH is a logarithmic scale used to specify the acidity or basicity of an aqueous solution. More precisely it is the negative of the base 10 logarithm of the activity(concentration) of the hydrogen ion (Bates, 1973). Determination of the pH value of meat is very important because it provides evidence as to how long the meat will keep and also its technical processing characteristics. The pH of a living muscle at rest ranges from 7.2 to 7.4, but in severe physical and mental stress, lactic acid is produced and pH may temporarily decrease to 6.2. In living animals, the accumulation of lactic acid causes pain and distress (Gregory, 1998). The ultimate pH is one of the most important indices of meat quality and together with meat colour, is normally used in standard evaluation of meat especially for ageing process (Węglarz, 2010). In chicken, the normal pH values at 15 minutes *post-mortem* (pH_{15}) are around 6.2 to 6.5, whereas normal ultimate pH (pHu) values are around 5.8. The acidification process depends on the rate of glycolysis in the muscle (Duclos et al., 2007) hence, muscle glycogen concentration at the time of slaughter is one of the most important factors affecting meat quality, including pH (Šimek et al., 2003).

Glycolytic potential provides a more accurate measure of glycogen level in the living muscle. After slaughter, the muscle converts glycogen into lactic acid and energy as fibres

attempt to keep the ATP content at the normal level (homeostasis). This lactate formation lowers pH (Puolanne et al., 2002). However, different muscles with the same lactate concentration may have different pH values possibly due to differences in buffering capacities and/or differences in the concentration of strong ions such as Mg^{2+}, Ca^{2+}, and Cl^{-} (van Laack, 2000). Light muscles (breast muscles) are known to possess better buffering capacities than dark muscles (leg muscles) due, chiefly to their higher content of histidine compounds (Decker, 2001). Reports also indicate that the pH value of muscles may be influenced by the presence of other acids such as free amino acids. The sum total of all acids in a meat sample constitutes the titratable acidity of meat (Šimek et al., 2003).

Intrinsic factors such as species, breed, muscle and animal variability and extrinsic factors (environmental temperatures) have also been reported to influence the rate and extent of pH decline during *rigor mortis*. According to Babji et al., (1982), heat stress of broilers increases the rate of *post-mortem* glycolysis and leads to pale meat colour with low pH; whereas muscles derived from cold-stressed birds normally show pH of about 6.6, have lower shear values and good meat quality characteristics. Research has also shown that the kind of diet the birds feed on can affect the glycolytic potential. According to Guardia et al., (2014), the breast muscle pH values were lower when broilers were fed on diets deficient in lysine but richer in other amino acids.

The effects of post slaughter factors such as meat thermal processing temperatures on pH have also been studied by some researchers. A study by Swatland, (1994), found that the pH of meat decreased by about 0.15 when the meat was warmed from 20 to 38^{o}C and increased by about 0.2 when the meat was cooled from 20 to 0^{o}C. On the other hand, Young et al., (1993), reported that the pH of pre-rigor muscle declines, whereas that of post-rigor muscles increases during cooking. This phenomenon suggests that when poultry meat is cooked before completion of the rigor process, there is sufficient pH change and the

temperature required for myoglobin denaturation increases above that normally achieved, so myoglobin is inadvertently protected. However, as Young et al., (1996) stated, myoglobin is less heat sensitive at its isoelectric point (PI), (pH ~ 7) than at remote pH values. This has a direct bearing on the colour of meat since pH controls the chemical state of myoglobin and the colour of meat is dependent on these states. The rate and extent of pH fall post-mortem also lowers the water holding capacity, the weight, saleability and economic value of the meat (Hughes et al., 2014).

3.2 Water holding capacity of poultry meat.

Water holding capacity (WHC) is the ability of a matrix of molecules, usually macromolecules present at low concentrations, to physically entrap large amounts of water in a manner that inhibits exudation (Fennema, 1996). Water is present in muscle to the tune of 75% at rigor. It is important for eating quality and economics. In meat, water improves palatability i.e. is responsible for the initial juiciness and tenderness (Aaslyng, 2002). Water also contributes to the economic yields of the products and provides for the water expected to be lost during cooking (Aaslyng, 2002). Being a universal solvent, water ensures dispersion and distribution of ingredients and is a critical protein solvent. Due to its high thermal capacity, water helps in temperature control that leads to improved protein solubility and control of bacterial activity.

Despite all these beneficial attributes, it is inevitable that water will always be lost from the carcass. This is a key concern for meat producers as it leads to diminished visual appeal and inferior palatability traits for consumers as well as reduced nutrient retention, protein functionality, and product yields for processors, thus affecting demand and saleable value (Mason et al., 2016). Since most meat products are sold on the basis of their weights, it stands to reason that loss of water in meat is directly proportional to a loss in revenue (Bowker & Zhuang, 2013; Mason et al., 2016). The water released can be described as drip,

purge, weep, exudate, or cook loss, and these are inversely related to water holding capacity (WHC) (Warner, 2014). WHC refers both to the bound water and also to water added during meat processing. Sometimes the term water-binding capacity (WBC) is used. However, WHC refers to the ability to bind water in raw meat, while WBC refers to the water bound by meat during its processing combined with heating (Pospiech & Montowska, 2011).

Most of the water in meat is held within the muscle cells (within the myofibrils, between the myofibrils, and between the myofibrils and the cell membranes), between muscle cells and between muscle bundles (Offer & Cousins, 1992). Within the myofibrils, it is found between the thick myosin and the thin actin filaments, particularly in the I- and not the more protein dense A-band. The size of this space varies with pH, sarcomere length, ionic strength, osmotic pressure and whether the muscle is pre- or post-rigor (Offer & Trinick, 1983).

Being dipolar, some water is normally bound to charged species like proteins. This is very small amount of water, 5-10 g/100 g of protein (Figure 3.1). This water is neither driven off by heating (100°C) or frozen at – 40°C/(-40°F). Bound water is no longer available as a solvent; is not driven off by application of forces or greatly affected by the conversion of muscle to meat (Pearce et al., 2011). Bound water attracts other water molecules, another 2-3 molecule layer around protein groups, 50-60 g/100 g proteins; this is immobilised water which accounts for up to 85% of myowater (Fennema, 1996). It is located within the thick filaments and between the thick and thin filaments of the myofibril (Honikel et al., 1986).

Immobilized water may be held either by steric effects and/or by attraction to the bound water. In early postmortem tissue, this water does not flow freely from the tissue, but can be removed by drying, or frozen at low temperatures. It is the most affected by the *rigor* process and the conversion of muscle to meat. Upon alteration of muscle cell structures and lowering of the pH, this water also escapes as purge (Huff-Lonergan & Lonergan, 2005).

The third form of water (~ 300 g/100 g protein), weakly attracted to the immobilized and bound water called free water (Figure 3.1). Free water exists in the sarcoplasmic area (sarcomeres) (Honikel & Hamm, 1987), in capillaries within the muscle cells, where it is held by intermolecular forces between the liquid and the surrounding matrix. Free water can be easily mobilized upon shrinking of myofibrils at the time of *rigor mortis* (Honikel, 2004).

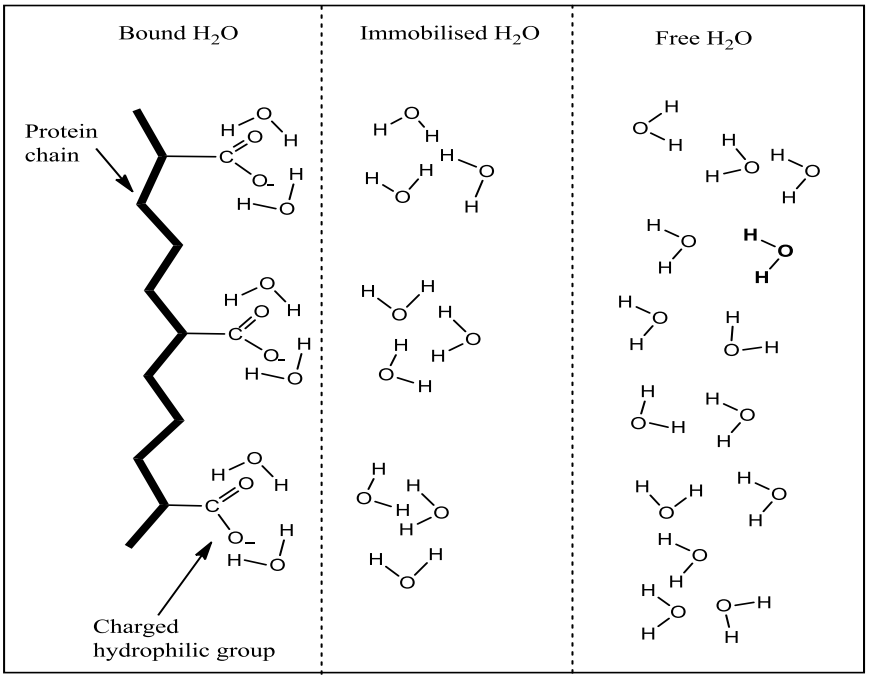


Figure 3. 1 Forms of water in muscles
Source: http://labs.ansci.uiuc.edu./meatscience/Library/free%20water20fig.gif

The distribution of the various forms of water in muscles and in meat is shown in Table 3.1.

Table 3. 1 Water distribution in muscle of live animal (pH ~ 7) and meat (pH 5.3 – 5.8)

	Water %	
	Muscle	Meat
Protein bound water	1	1
Intramyofibrilar	80	75
Extramyofibrilar	15	10
Extracellular	5	15

Adopted from Honikel, (2009)

Thus knowledge of factors that influence the WHC of meat is of considerable economic interest. Moreover, investigations into the causes of changes in WHC of meat teach us about alterations in, especially myofibrillar muscle proteins, which play the most important role both in the function of the muscles and also in their WHC (Honikel, 2004). Some of the factors that affect WHC of muscle and meat include the diet and genotype, muscle structures, stress and pH fall, and the presence of ions (Honikel, 2004).

During growth and development, the genotype and diet of meat animals have direct influence on muscle characteristics with diet directly affecting the intramuscular fat levels in the animal body. Research has shown that muscles having higher contents of intramuscular fat tend to exhibit higher water-holding capacities. The reasons for this effect are unknown; possibly the intramuscular fat loosens up the microstructure, thus allowing more water to be entrained (Lawrie & Ledward, 1985).

In the living animal, water is kept in cells by the sarcolemma and is maintained by various membrane pumps. Post-slaughter, water is moved to the sarcoplasm from the intramyofibrillar space, by shrinkage of the myofibrils, and kept in the cell until the pH falls, ATP becomes unavailable, and water and ions can pass through the sarcolemma into the extracellular spaces between the cells. In muscle of a live animal (pH ~ 7), the myofibrils take up most of the space in the muscle cell and more than 95% of the water is within cells. Postmortem, the myofibrils shrink, the intracellular sarcoplasmic space increases, and about 15% of the water moves to the extracellular space. This water appears as drip at the surface of the meat (Hughes et al., 2014).

From a structural perspective therefore, water loss from meat is mainly influenced by; (1) the longitudinal and transverse shrinkage of myofibrils, which changes interfilament spacing, (2) breakdown in the cell membrane structure making it more permeable to water, (3) integrity of the intracellular cytoskeleton, which affects shrinkage of the whole cell, (4)

development of spaces between cells allowing fluid to accumulate and flow (Hughes et al., 2014), and (5) development of a network between sarcoplasmic and myofibrillar proteins, which entraps water (Liu et al., 2016).

In the pre-slaughter period, stresses on the animal such as fasting, and different stunning methods are likely to influence meat WHC (Cheng & Sun, 2008). Stress is associated with postmoterm anaerobic muscle glycolysis which directly affects the rate and extent of pH fall; major determinants of the WHC of raw, processed, and cooked meat products. Associated with the fall in pH in "normal" muscle post-mortem (7.0 to 5.5) is the shrinkage in the myofilament lattice and the exudation of water. During rigor, as the pH of the muscle approaches 5.4 or lower, the net charge on proteins in the myofibrils diminishes and filaments can approach each other, causing transverse shrinkage in the myofibril (Figure 3.2). Thus, there is always some loss of WHC when the pH of muscle drops from 7.0 to 5.5. However, meat of high ultimate pH [dark firm, and dry (DFD)] does not undergo shrinkage in the myofibrils and muscle cells postmortem. In contrast, meat of low ultimate pH with denatured proteins [pale, soft, and exudative (PSE)] has excessive shrinkage in the myofibrils and muscle cells.

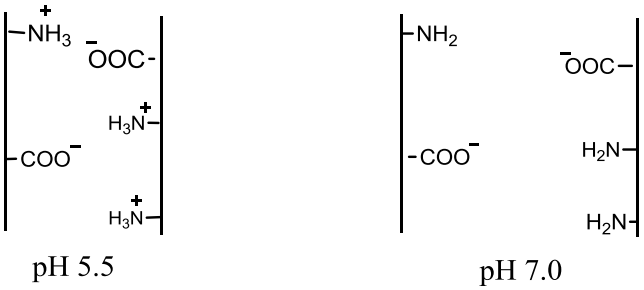


Figure 3. 2 Schematic of protein shrinkage by changes in pH
From Honikel, (2009).

The isoelectric point, defined as the point of minimum charge, of the myofibrillar proteins occurs at pH 5.0 - 5.2. As the ultimate pH approaches the isoelectric point of the

muscle proteins, the WHC of the muscle reaches a minimum. Meat that experiences a large pH fall (7.0 – 5.3; pHu 5.3), will lose more fluid and has lower WHC. Conversely meat that has only a small drop in pH (pHu > 5.8); loses less fluid and is described as DFD. This condition is mostly caused by low muscle glycogen content at slaughter as a result of preslaughter stress. In meat prone to PSE conditions, high temperatures (HTs) shortly after slaughter (35 – 42^{o}C) and the low pH due to rapid glycolysis, denatures myosin and destroys membranes. The denaturation causes a shrinkage of the myosin head from 19 nm to 17 nm in addition to the shrinkage due to the low pH (Offer & Knight, 1988).

A faster rate of pH fall speeds up contraction of actomyosin as it forms and thus exudes bound water. At high temperatures the loss of WHC is always partly due to increased denaturation of the muscle proteins, and partly to enhanced movement of water into extracellular spaces (Lawrie & Ledward, 1985). WHC post-slaughter may also be affected by chilling, ageing, injecting non-meat ingredients, as well as tumbling. Furthermore, the cooking and the cooling methods, the heating and the cooling rate, the cooking and the end point temperatures have effects on the WHC (Cheng & Sun, 2008). Lesiak et al., (1996), studied the effect of post-mortem temperature (0, 12, and 30^{o}C) and time on the WHC of hot boned turkey breast and thigh muscles. They reported that higher temperatures and longer storage time induced greater drip losses in the breast muscles. Longer storage time induced greater drip losses but least drip loss occurred at 12^{o}C in thigh muscle.

The impact that various ions have on proteins and their ability to bind water is described by the Hofmeister series. According to the series, the anions have stabilization abilities that vary in the order; $PO_4^{3-} > SO_4^{2-} > CH_3COO^- > Cl^- > Br^- > NO_3^- > I^-$. For the cations the series is $(CH_3)_4N^+ > NH_4^+ > K^+ > Na^+ > Mg^{2+} > Ca^{2+}$. In addition, the mechanism by which various ions increase WHC can be described by classifying the ions into chaotropic compounds, that destabilize the proteins native structure, and kosmotropic ions that stabilize

the protein structure. Strong chaotropic cations, such as K^+ and NH_4^+, and weak kosmotropic anions, such as carboxylic groups of amino acids, stabilize biological systems, and increase WHC of meat (Puolanne & Halonen, 2010).

Ionic strength influence myofibrillar protein solubility, WHC, and other physico-chemical and functional properties of foods; such as their emulsifying, foaming, gelling properties, and viscosity. Myofibrillar proteins are more soluble at higher ionic strength (0.2 - 0.6 M) (Chen et al., 2015; Trout & Schmidt, 1987) with variations dependent on pH, which can be influenced by the phosphate used. Only 50% of myofibrillar proteins are soluble at ionic strengths < 0.2 M (Chen et al., 2015). High ionic strength extraction solutions will dissociate the actomyosin complex more completely, resulting in higher mole ratios of actin and myosin than lower ionic strength solutions. Commercially, a high ionic strength (0.47-0.68 M (2% - 3% salt)) is required to fully develop the functional properties of muscle tissue foods (Chen et al., 2015). However, at high ionic strength, salt has a dehydrating effect; thus a decrease in WHC is observed at ionic strength >1.0 and a NaCl addition of 5% with no added water, and 8% NaCl with 60% water added (Hamm, 1986). A maximum in WHC of muscle tissue is observed at ionic strength about 0.8 - 1.0, due to the initiation of the "salting out" of the myofibrillar proteins, which become insoluble at >1.0. Thus in the salting and curing of meat products, it is important to ensure that this ionic strength and salt concentration is not reached, in order to ensure maximumWHC (Knight & Parsons, 1988).

Knight & Parsons, (1988) attributed the "salting out" effect to entropic swelling pressure caused by a steric resistance to the rotational movement of the tails of myosin molecules imposed by the actin filaments to which they get attached. The swelling was greatest in w 6% NaCl. They attributed the apparent dehydrating effect of higher salt concentrations to the precipitation of myosin, a feature that would reverse its depolymerization in sodium chloride and cause shrinkage.

Water-holding assessment procedures include the filter paper press methods (Grau & Hamm, 1953), centrifugation methods (Honikel & Hamm, 1987), and suction loss methods (Fischer et al., 1976). With these methods the amount of water released is far higher than with methods without external force as the pressure applied enforces the release of water from the intra- and extracellular space of the muscle structure. In drip loss measurements only extracellular water exudes from the meat. Therefore, a factor must be known to evaluate the actual drip loss of the meat (Honikel, 2004).

3.3 Lipid oxidation

Poultry meat just like any other tissues rich in lipids, especially polyunsaturated fatty acids experiences oxidation of the fats into short-chain breakdown poducts, chiefly aldehydes, alcohols, alkanes, ketones and ethers which are objectionable in taste and colour and lead to rancidification. The nutritional value of meat is reduced and vitamins are susceptible to degradation if rancidification occurs. Toxic compounds leading to long-term harmful health effects may also be produced. The susceptibility of meat to oxidation is higher when the poultry meat enriched with PUFA contains longer FA with a higher number of double bonds; thus, fatty acid make-up of oil has a major effect on its stability and flavour quality.

Fatemi & Hammond, (1980), measured the relative rates of oxidation of the pure oleate (18:1), linoleate (18:2), and linolenate (18:3) fatty esters as 1:10.3:21.6. However, it is difficult to predict the contribution of different fatty acids in promoting oxidation when present in mixtures as is the case in natural fats. Some studies showed significant interactions between different unsaturated fatty esters. Despite that however, lipid oxidation generally causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce shelf life (Cortinas et al., 2005).

Lipid peroxidation is a complex process known to occur in both plants and animals. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a

rearrangement of the double bonds in unsaturated lipids to make the double bonds along the chain conjugated and the eventual destruction of membrane lipids, with the production of a variety of breakdown products as earlier mentioned (Dianzani & Barrera, 2008).

In pathological situations reactive oxygen and nitrogen species are generated at higher than normal rates, and as a consequence, lipid peroxidation occurs with α-tocopherol deficiency. In addition to containing high concentrations of polyunsaturated fatty acids and transition metals, biological membranes of cells and organelles are constantly being subjected to various types of damage (Halliwell & Gutteridge, 1984). Lipid oxidative processes are faster in the presence of factors such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavours and loss of essential amino acids, fat-soluble vitamins, and other bioactives. So, the process may be categorised as auto, photo, thermal, and or enzymatic oxidation under different conditions, most of which involve some types of free radicals or reactive oxygen and nitrogen species (Bigolin et al., 2013).

Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids (Shahidi & Zhong, 2005). Classical studies established that the mechanism of autoxidation of lipids is a chain reaction involving the three stages of initiation, propagation and termination as demonstrated in Figure 3.3 (Kanner & Rosenthal, 1992). The initiation step starts by the abstraction of the labile hydrogen positioned α to the double bond, by a radical of sufficient reactivity. In autoxidation of lipids in biological systems, the formation of the free radical is a direct process that occurs by homolysis of endogenous hydroperoxides by scission of ROOH and production of RO• and •OH, one of which abstracts the labile hydrogen leading to formation of a lipid radical. This step requires a relatively high activation energy, which may be provided, for example by heat energy or singlet oxygen (Dąbrowska et al., 2015).

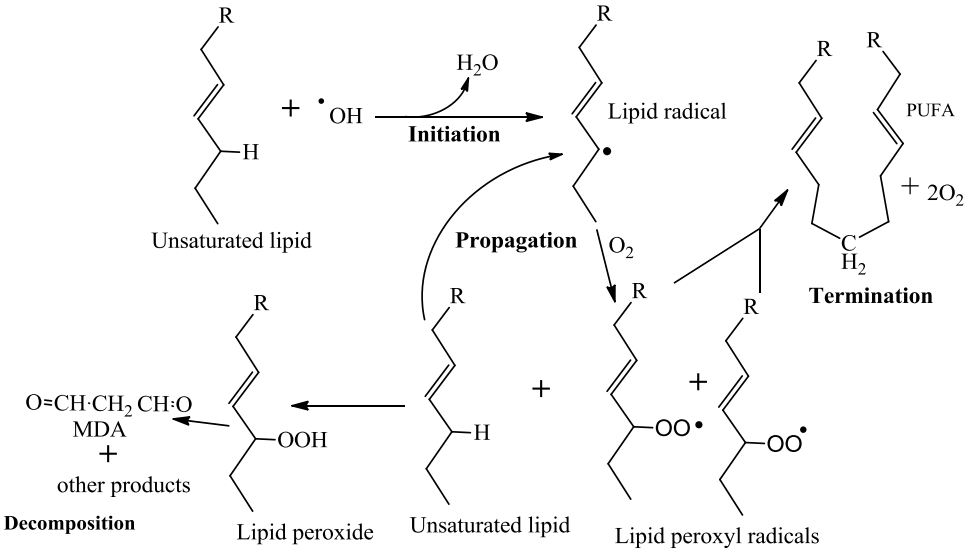


Figure 3. 3 Radical chain reaction mechanism of lipid peroxidation.
As modified from the work of Tim & Young McEneny, (2001).

In the propagation phase, the lipid radical rapidly reacts with oxygen to form a lipid peroxy radical which abstracts a hydrogen from another lipid molecule generating a new lipid radical (that continues the chain reaction) and a lipid hydroperoxide (Ayala et al., 2014). The last step of lipid peroxidation is a termination process in which two of the hydroperoxyl radicals react with each other, with another radical and/or each undergoes self destruction to form non-radical products. Although the hydroperoxide molecules formed at the primary stage are stable at physiological conditions, they can be decomposed by heating at high temperature or by exposure to transitional metal ions (Min & Ahn, 2005). They decompose to form volatile aromatic compounds, which are perceived as off-flavours and as a warning that food is no longer fit for human consumption (Wąsowicz et al., 2004).

Several of these decomposition products have been reportd including the aldehydes; propanal, hexanal, 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) (Ayala et al., 2014). Among them, MDA which is an end-product generated by decomposition of arachidonic acid and larger PUFAs, through enzymatic or nonenzymatic processes has extensively been studied. Its levels in biological systems have been used as an indication of

lipid oxidation (Singh et al., 2014). According to Ayala et al., (2014), MDA is an important contributor to DNA damage and mutation and its amount generated during lipid peroxidation can be measured by the TBARS (Thiobarbituric Acid Reactive Substances) method, commonly used due to its simplicity and cheapness. It is based on the ability of MDA to react with thiobarbituric acid (TBA) (Sochor et al., 2012). Lipid oxidation affects shelf life; the time period for the product to become unacceptable from sensory, nutritional or safety perspectives. It also brings about the warmed over flavour (WOF); the rancid flavour developed during storage under refrigeration brought about by rapid increase in oxidation of cooked meat products. According to Lage et al., (2012), the cooking temperature, time, and final internal temperature of the meat can influence the development of WOF. The effects of such cooking parameters are related to differences in the formation of Maillard reaction products (MRP) in the meat.

Literatures have revealed the ability of metals such as iron, copper, cadmium, chromium, lead, mercury, nickel, and vanadium to produce reactive oxygen species, resulting in lipid peroxidation, DNA damage, depletion of sulfhydryls, and altered calcium homeostasis. However, comparative evaluation has revealed that Fe (II) is a more effective catalyst of lipid oxidation than the other mentioned ions. Further, it has been shown that lipid peroxidation requires both Fe (III) and Fe (II), probably as a dioxygen-iron complex. Most iron is complexed, and little free iron actually exists in nature (Stohs & Bagchi, 1995).

In humans, the manner in which chronic ethanol consumption may induce hepatic injury is still an enigma but lipid peroxidation and generation of free radicals due to ethanol consumption have been hypothesized as possible mechanisms. The increased lipid peroxidation observed after acute (Lrrov et al., 1978) and chronic ethanol ingestion lends support to this theory. Furthermore, increased activity of free radicals and enhanced lipid peroxidation in the liver of alcoholics have been reported (Suematsu et al., 1981).

3.4 Microbiological aspects of meat

The muscles of healthy animals kept under good husbandry conditions are usually sterile, but the slaughtering process may provide bacteria with an opportunity to colonize meat surfaces. Microbiological contamination is reportedly more common than both microphysical and chemical contaminations. The relative number of illnesses due to food-borne microorganisms makes microbiological quality an important food safety factor (Alum et al., 2016). People become infected by consuming inadequately cooked poultry or other foods that become cross-contaminated via contact with poultry. There are many potential sources of meat contamination by microorganisms, for example the contents of the gastrointestinal tract, (released during dressing operations), airborne contamination, aqueous sources, various vessels and receptacles, and the personnel, and also by cross-contamination from carcass to carcass (Mohamed-Noor et al., 2012). Broiler breast meat characterized by high pH (> 6.0) is more succeptible to microbial growth that can impair taste, flavour and appearance (Petracci et al., 2015). Further, phagocytic granulocytes undergo respiratory burst to produce oxygen radicals to destroy the intracellular pathogens but which can initiate peroxidation of lipids, destroy healthy cells and bring about meat spoilage (Robinson, 2008).

The most important factor in chicken meat quality is prevention from microbial contamination. This requires an integrated approach, along the poultry production chain which includes the washing of hands, wearing of protective clothings and dedicated footwears, house cleaning and disinfection and the removal of spent litter between two flocks (Vandeplas et al., 2008). Care should also be taken to lower the risk of contamination with faeces prior to slaughter, and while emptying the intestinal tract during dissection (Haslinger et al., 2007). Post-slaughter, contamination can be reduced using methods such as cooling, vapour-vacuum system, vapour pasteurization and chemical treatment with chlorine and chlorine compounds, ozone, organic acids, trisodium phosphate (Canan et al., 2007).

Chapter 4

POULTRY MEAT PROCESSING, PAH GENERATION AND FOOD SAFETY

4.0 Introduction

Poultry meat consumption has increased tremendously over the past decades and chicken meat alone constitutes over 30% of the global meat consumption. This is associated with the meat's low fat and high PUFA contents, rise in income per capita, urbanization, and related attributes. Before poultry meat is consumed it has to undergo alimentary processes to ensure that the meat is in the right state for consumption. In these preparative stages the meat is normally grilled, roasted, fried, toasted, boiled or barbecued. These alimentary processes help destroy harmful microorganisms, increase digestibility of proteins and raise organoleptic profiles; but may also lead to formation of carcinogenic substances principally, polycyclic aromatic hydrocarbons (PAHs) and or heterocyclic aromatic amines (HCAs), both of which are reported carcinogens (Kizi et al., 2011; Lynnette, 2010).

The amount of these carcinogenic compounds formed has been reported to depend, among others, on the high temperature employed during processing (Deshpande, 2002). Moreover, many consumers prefer well-done, strongly heated meat and meat products that may be heavily laden with the carcinogens, thus affecting food safety (Jägerstad & Skog, 2005). PAHs are ubiquitous environmental contaminants mainly formed by the incomplete combustion of organic matter, through the Maillard reaction that employs free radical mechanisms at some stages. Such compounds cause different types of DNA damage: nucleotide alterations and gross chromosomal aberrations. Most genotoxic compounds begin their actions at the DNA level by forming carcinogen-DNA adducts, which result from the covalent binding of a carcinogen or part of a carcinogen to a nucleotide. The most prominent members of these carcinogenic compounds contain aromatic nuclei (Jägerstad & Skog, 2005).

4.1 Aromaticity and aromatic compounds

Aromaticity is a chemical property in which a conjugated ring of unsaturated bonds, lone pairs, or empty orbitals exhibits stabilization stronger than would be expected by the stabilization of conjugation alone. Thus it is a manifestation of cyclic delocalization and of resonance. The first known use of the word "aromatic" as a chemical term was applied by August Wilhelm Hofmann in 1855 to compounds that contain the benzene groups. However, Hofmann said nothing about why he introduced an adjective indicating olfactory character to a group of chemical substances, only some of which have notable aromas (Jochen, 2009). The simplest and yet the most notable aromatic compound, first discovered by Michael Faraday in 1825 is benzene. The structure of benzene remained for a long time a centre of dispute in the scientific community until its cyclohexatriene structure (Figure 1.a) was first proposed by August Kekulè in 1865.

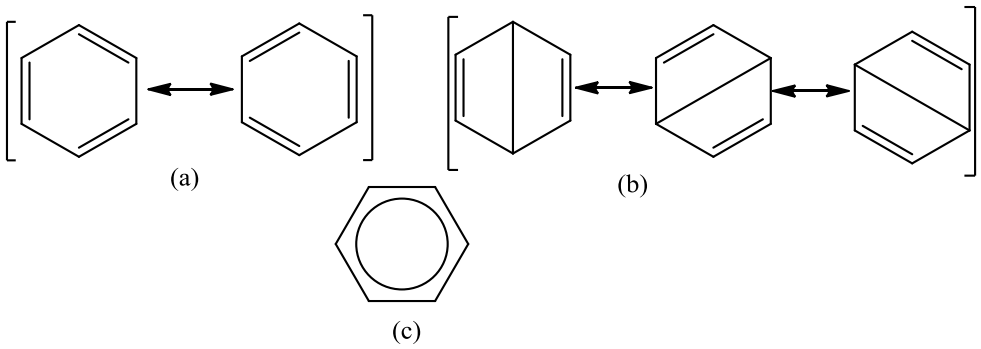


Figure 4. 1 Proposed resonance structures of Benzene
(a) Kekulé, (~ 80%), (b) Dewar (~ 20%) and (c) Robinson (Peter, 1977).

The key aromatic compounds of commercial interest are benzene, toluene and ortho- and para-xylenes. About 35 million tonnes of these compounds are produced worldwide every year. They are extracted from complex mixtures obtained by the refining of oil or by distillation of coal tar, and are used to produce a range of important chemicals and polymers, including styrene, phenol, aniline, polyester and nylon (Sainsbury, 1992). Aromatic compounds can usually be classified into three types:

- *Substituted benzenes***:** compounds containing simple benzene rings in their structures. Examples include trinitrotoluene (TNT), acetylsalicylic acid (aspirin), 1,3-benzodioxole (methylenedioxybenzene) and paracetamol.
- *Heterocyclics:* compounds where one or more of the atoms in the aromatic ring is of an element other than carbon. This can alter the ring's aromaticity, and thus (as in the case of furan) change its reactivity. Other examples include pyridine, imidazole, pyrazole, oxazole, thiophene, and their benzannulated analogs (Tewari et al., 1998)
- *Polycyclic aromatic hydrocarbons:* molecules containing two or more simple aromatic rings fused together by sharing two neighbouring carbon atoms such as naphthalene, anthracene and phenanthrene (Tewari et al., 1998)

4.2 Polycyclic aromatic hydrocarbons (PAHs)

4.2.1 Historical perspectives of PAH carcinogenicity

Polycyclic aromatic hydrocarbons (PAHs), which were first discovered in coal tar in the 19th Century, have become one of the most widely investigated compounds in medical sciences, biology, organic chemistry, physics and material sciences in recent years (Dias, 1988; Harvey, 1997). PAHs were the first chemical carcinogens to be discovered when in 1775, the English surgeon P. Pott of St Bartholomew's hospital, London, found an association between exposure to soot and a high incidence of scrotal cancers in chimney sweepers (Simko, 2002). The famous description of chemically induced carcinogenesis found the experimental counterpart in the coal tar tumours induced in rabbits by Yamagiwa and Ichikawa in 1915 (Pitot, 1990, cited in Wang & Yu, (2005). The carcinogenic potential of PAHs was recognized in 1930 when Kennaway and Hieger isolated, the first pure chemical carcinogen, Dibenzo (a) anthracene (DaA), from soot extract at the Chester Beatty Research Institute and determined its carcinogenicity (Kennaway and Hieger, 1930, cited in Wang & Yu, (2005). After further research on coal tar, Cook and friends discovered Benzo (a) Pyrene

(BaP), as one of the major carcinogenic components in 1933. Later research indicated that it was PAHs in the residues of combustion such as soot and coal tar which caused skin cancers of humans and animals. PAHs were regarded as the main carcinogens before 1950s.

In 1953, Doll, using wide epidemiological and statistical analyses proved that cigarette smoking was a prime cause of lung cancer. Careful analysis of the smoke and tar from cigarettes proved it contained many carcinogenic PAHs, from which BaP was assessed as the most dangerous compound (Simko, 2002). Since then, epidemiological studies on PAHs, have labeled soot, coal tar, and pitch as human carcinogens (Grimmer et al., 1988). Moreover, the higher incidences of respiratory tract and upper gastrointestinal tract tumours were also associated with occupational exposures to PAHs (Dipple, 1985). The high incidences of stomach cancer among populations in Nigeria (Alonge, 1988) or in the Baltic countries (Dungel, 1961) were related to the large consumption of fish smoked by artisanal wood combustion procedures generating high PAH levels. The very high prevalence of lung cancer among women in China and Taiwan was probably due to absorption of smoke from heated oils in the kitchen (Gao et al., 1987).

Nowadays, PAHs are still one of the most important classes of carcinogens due to their abundance in the environment (Fetzer, 2000; Luch, 2005). PAHs are also found in the interstellar medium, comets and meteorites. A team led by A. Witt of the University of Toledo, Ohio studied ultraviolet light emitted by the Red Rectangle nebula and found the spectral signatures of anthracene and pyrene. This discovery was considered as the confirmation of the PAH world hypothesis. This biological hypothesis proposes that PAHs served as the basis for the origin of life in a pre-RNA world (Cleaves & Chen, 2006).

4.2.2 Areas of application of PAHs

To the interest of organic chemists and material scientists, the most attractive property of PAHs is their aromaticity. The electron delocalization along the polycyclic aromatic

structures gives rise to interesting electronic and optical properties of these PAH materials. The breakthrough discovery of conducting and semiconducting organic polymers in 1970s leads to promising applications in the field of organic electronics nowadays (Wong, 2015). Their intrinsic electronic properties and the versatile functionalization qualified PAHs as other promising semiconducting materials in organic devices such as light-emitting diodes (LED), field effect transistors (FET), liquid crystal display (LCD) and solar cells (Hutten, 2000). On the other hand, these polycyclic aromatic molecules can form stable columnar mesophase after attaching flexible chains, which are desirable for device processing due to their self-assembly and self-healing capability (Bushby & Lozman, 2002; Sage, 1998).

Furthermore, two-dimensional all-benzenoid PAHs are viewed as model compounds for graphite. Therefore, PAHs are also of special interest in theoretical problems like the scope, limitation and effects of electron delocalization in aromatic materials (Dias, 1987).

4.2.3 Mechanism of PAH formation during grilling of meat

Although the exact mechanism of PAH formation is not well understood, some authors postulated that they might be formed through free radical reactions, intramolecular addition or polymerization of small molecules (Perez et al., 1986). They also reported that thermal degradation of steroids such as cholesterol and androsterone may be responsible for the formation of mutagenic and carcinogenic PAHs such as Phenanthrene, Fluoranthene and their derivatives. Some authors like Manahan, (1994) proposed that PAHs may be synthesized from saturated hydrocarbons under oxygen-deficient conditions. Pyrosynthesis and pyrolysis are two main mechanisms that can explain the formation of PAHs. Low molecular weight hydrocarbons form PAHs by pyrosynthesis (Chen & Chen, 2001).

When the temperature exceeds 500 ^{o}C, carbon–hydrogen and carbon–carbon bonds are broken to form free radicals. These radicals combine to form acetylenes which further condense with aromatic ring structures, which are resistant to thermal degradation. According

to Deshpande, (2002) the formation of pyrogenic compounds is a complex process and differs from other heat induced processes in that the former is preceded by an initial extensive breakdown of the molecular structures of organic compounds to simpler reactive fragments. Combination of these fragments follow provided the conditions preclude rapid formation of CO or CO_2. PAHs are most likely pyrosenthesised from degradative products consisting of 4 or 2 units such as butadienes or ethylene radicals (Wynder, 1972, as cited in Deshpande, 2002). The ease of their formation at elevated temperatures follows from their thermodynamic stabilities. Some possible pathways showing the formation of benzo[a]pyrene are shown in Figure 4.2

Three possible mechanisms were proposed by Haynes, (1991) for PAH formation during combustion: (1) Slow Diels–Alder condensations, (2) Rapid radical reactions, and (3) Ionic reactions. However, the radical formation mechanism is favoured as the combustion process within the internal combustion engine has to occur very rapidly. It seems that gaseous hydrocarbon radicals rearrange quickly, providing the mechanism of PAH formation and growth. The addition of hydrocarbon radicals to lower molecular weight PAHs then leads, via alkyl PAHs, to the formation of higher PAHs (Wiersum, 1996 as cited in Ravindra et al, (2006). A study by Atal et al., (1997) suggests that pyrosynthesis is the major mechanism for PAH emissions from combustion processes. The existing ring structure of cyclic compounds is conducive to PAH formation. Unsaturated compounds are especially susceptible to the reactions involved in PAH formation. The higher alkanes present in fuels and plant materials form PAHs by the process of pyrolysis of organic compounds (Atal et al., 1997). The proposed mechanism also explains the observation that during hot smoking of mainly protein containing products, the incomplete combustion of fat in the products generates PAHs due to pyrolysis (Atal et al., 1997).

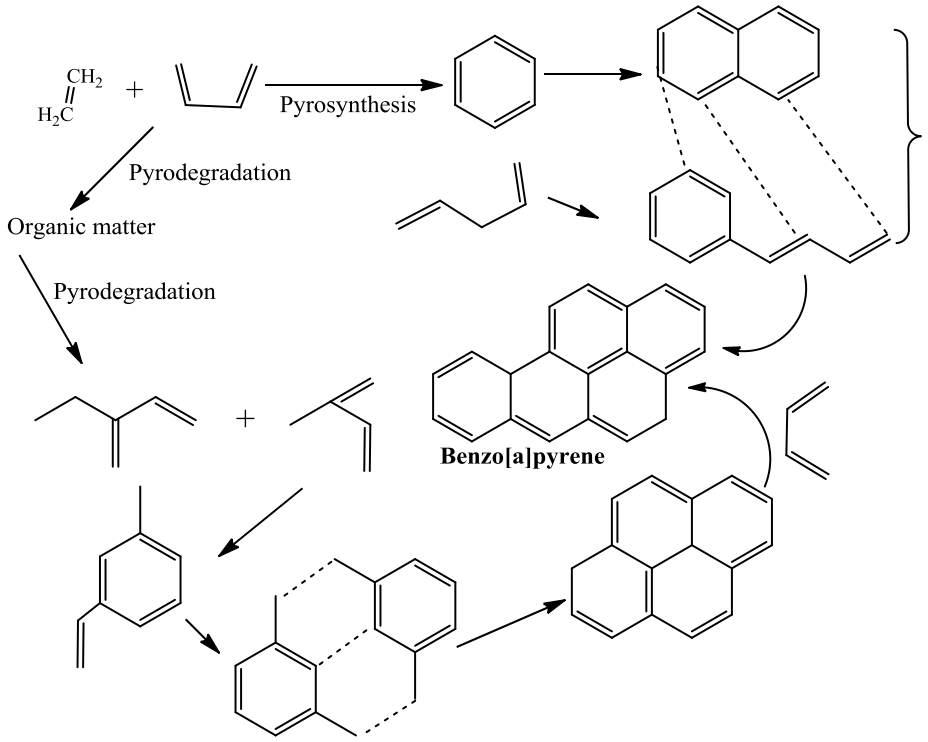


Figure 4. 2 Possible Pathways for the formation of BaP
Adopted from Deshpande, (2002).

The tendency of hydrocarbons to form PAH structures by pyrosynthesis varies in the order; aromatics > cycloolefins > olefins > paraffin (Manahan, 1994).

4.2.4 Properties of PAHs

Five properties in particular influence the biological activity of PAHs; their vapour pressure, their adsorption onto surfaces of solid carrier particles, their absorption into liquid carriers, their octanol-water partition coefficient in tissues and their limits of solubility in the lipid and aqueous phases of tissues (IARC, 2010). These characteristics determine their capacity for transport and distribution between different environmental compartments and their uptake and accumulation by living organisms. The transportation of PAHs in the atmosphere is influenced by their volatility. The chemical reactivity of PAHs influences adsorption to organic materials or degradation in the environment. All these factors determine the persistence and capacity of PAHs to bioaccumulate in the food chain (SCF, 2002).

4.2.5 Priority Polycyclic Aromatic Hydrocarbons

USEPA has listed sixteen PAHs as priority pollutants. The molecular structures and names of these compounds are shown in Figure 4.3. These have always been the most commonly studied PAHs.

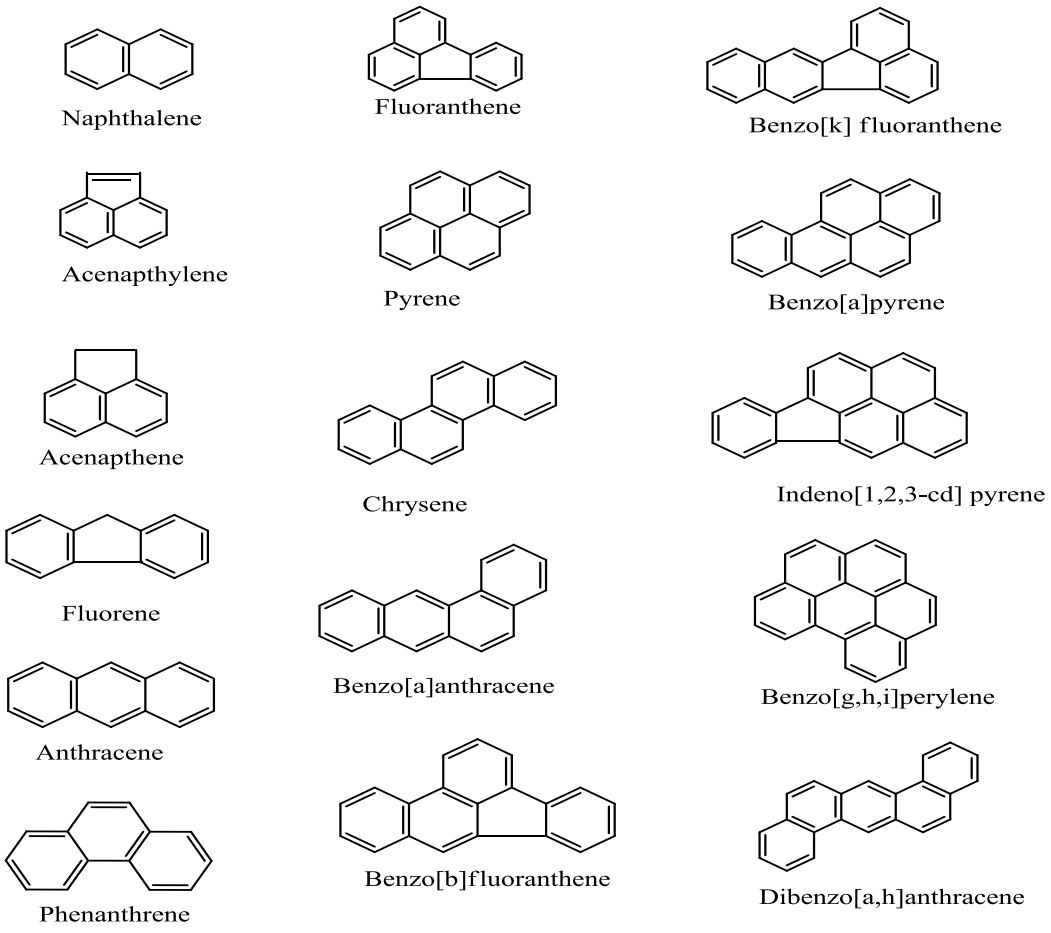


Figure 4. 3 Molecular structures of the 16 priority PAHs
From Mottier et al., (2000)

4.3 Human exposure to PAHs

Humans are exposed to PAHs through inhalation, ingestion and dermal contact with food alone constituting over 90% exposures in nonsmokers (Menzie et al., 1992). Food products can be contaminated through various ways including grilling (Chung et al., 2011). Smokers consuming one pack of non-filtered cigarettes per day have an estimated additional intake of 1-5 μg /day (Hattemer-Frey & Travis, 1991). People working with creosote or

asphalt and using coal tar-based anti-dandruff shampoos are also exposed to PAHs. Drinking contaminated water or cow's milk and breast feeding constitute other exposure routes (ATSDR, 1995a). The oral exposure of lactating goats to PAHs was found to result in a constant level of native PAH forms and in a significant increase in monohydroxylated metabolites in milk (Grova et al., 2006). It is also reported that PAHs may cross the placenta and enter the body of the unborn foetus (ATSDR, 2009).

Studies in USA on men aged between 19 and 50 years indicated that, in nonsmokers a mean total intake of 3.12 mg/day was estimated, of which food contributed 96.2%, air 1.6%, water 0.2% and soil 0.4% (Hattemer-Frey & Travis, 1991). Food products on the other hand can be contaminated through contaminated soils and packing materials, polluted air and water, and alimentary processes (Chung et al., 2011)

4.4 Meat consumption and carcinogenesis

When meat is exposed to naked flame, pyrolysis of fats in the meat generates PAHs that can be deposited on the meat. Even if the meat is not directly cooked over a flame, fat dripping onto the fire undergoes pyrolysis to form PAHs such as BaP. The PAHs formed rise with the smoke to adhere to the surface of the meat. Correspondingly it has been shown that methods of cooking in which the heat source is above the meat or that involve separation of meat from smoke will result in lower PAH formation. Reports indicate that PAH formation in cooked meat is a function of: 1) cooking temperature and time, 2) levels of doneness, 3) fat content of meat, 4) proximity to and the type of heat source, and 5) the smoking process used (Jägerstad & Skog, 2005).

Numerous epidemiologic studies have used detailed questionnaires to examine participants' meat consumption and cooking methods (Abid et al., 2014). A summary of studies regarding the potential adverse health effects of red meat consumption on major chronic diseases, such as diabetes, coronary heart disease, heart failure, stroke and cancer at

several sites, and mortality have been reported (Wolk, 2017). Summary results of risk estimates from pooled analyses and meta-analyses together with recently published findings based on at least six cohorts, for the consumption of unprocessed red meat of 100 g day^{-1} varied from nonsignificant to statistically significantly increased risk (11% for stroke and for breast cancer, 15% for cardiovascular mortality, 17% for colorectal and 19% for advanced prostate cancer); for the consumption of 50 g day^{-1} processed meat, the risks were statistically significantly increased for most of the studied diseases (4% for total prostate cancer, 8% for cancer mortality, 9% for breast, 18% for colorectal and 19% for pancreatic cancer, 13% for stroke, 22% for total and 24% for cardiovascular mortality and 32% for diabetes)(Wolk, 2017). A WHO, (2015), report indicates that processed meat was classified as carcinogenic to humans (Group 1), based on sufficient evidence in humans that the consumption of processed meat causes colorectal cancer.The report further concluded that each 50 gram portion of processed meat eaten daily increases the risk of colorectal cancer by 18%.

On the contrary, reports indicate that white meat (poultry, fish) are not associated with risk of cancer. In substitution models with total meat intake held constant, a 10 gram increase in white meat intake offset by an equal decrease in red meat intake was associated with a statistically significantly reduced (3–20%) risk of cancers of the esophagus, liver, colon, rectum, anus, lung, and pleura (Carrie et al., 2012). In addition models with red meat intake held constant, poultry intake remained inversely associated with esophageal squamous cell carcinoma, liver cancer, and lung cancer, but mixed findings were observed for fish intake. As the dietary recommendations intend, the inverse association observed between white meat intake and cancer risk may be largely due to the substitution of red meat (Carrie et al., 2012).

In another study,Wilson et al., (2016), reported that substituting 30 g day^{-1} of poultry or fish for total or unprocessed red meat was associated with significantly lower risk of recurrence of prostate cancer. Lower intakes of red meat and well-done red meat and higher

intakes of poultry and fish are associated with lower risk of high grade and advanced prostate cancer and reduced recurrence risk, independent of stage and grade. Further, a study by Joshi et al., (2015) support the role of specific meat types and cooking practices as possible sources of human carcinogens relevant for colorectal cancer (CRC) risk. On the other hand, a study by Wang et al., (2014) reported that the gene GSTP1 Ile105Val might modify the association between intake of poultry cooked with high temperature methods and CRC risk ($p = 0.0035$), a finding that was stronger among rectal cancer cases. Their findings therefore suggest a possible role for diets high in poultry cooked at high temperatures in CRC risk.

4.5 Toxicokinetics of PAHs in mammals

Studies have been carried out to determine the entry routes, the routes followed while in the organisms, the fates of the compounds, the rate and effects of these changes in the body and how the products of the changes leave the body. A brief review of these is here given.

4.5.1 Absorption

Absorption of PAHs from the diet is determined by the size and lipophilicity of the molecule, the presence of bile in the digestive tract, the dose ingested and the lipid content of the diet. PAHs are highly lipid-soluble and are absorbed from the lung, gut and skin of mammals. Studies on the lung retention of microcrystalline PAHs or PAHs in solution after intra-tracheal instillation in female rats have indicated that about 35- 99% is cleared from the respiratory tract within 2-4 hours (ATSDR, 1996). However, inhaled PAHs are predominantly adsorbed on soot particles which can be eliminated from the airwaves by bronchial clearance. However, PAHs might be partly removed from the particles during transport on the ciliated mucosa and may penetrate into the bronchial epithelium cells where metabolism takes place.

BaP and other PAHs are known to readily absorb from the gastrointestinal tract when present as solutes in various dietary lipids. The absorption is facilitated by the presence of bile

salts in the intestinal lumen. Studies in rat showed that more than 30 -50% of low oral doses of BaP or pyrene were readily absorbed and a major part was rapidly metabolised in the liver (Withey, et al, 1991, as cited in WHO, (2000). Studies have also shown that, irrespective of the route of administration PAHs are widely distributed in the organisms with the highest concentrations in the liver. Mammary and other fatty tissues are significant storage depots but owing to the rapid metabolism, accumulation is always low (WHO, 2000).

4.5.2 Metabolism

Under physiological conditions, PAHs themselves are inert towards biological systems being readily metabolized by organs such as the liver, kidneys, testes, lungs, intestines, thyroid, adrenal and sebaceous glands (Ramesh et al., 2001). However, the metabolic transformation of PAHs may lead to carcinogenic, mutagenic and tumorigenic intermediates. The absorbed PAH is metabolized to increase its solubility and excretion from the system. In total, three principal metabolic pathways have been described for PAHs (Xue & Warshawsky, 2005). Historically, the bay region dihydrodiol epoxide pathway (P450 1A1/1B1-monooxygenase dependent) was the first discovered as being crucial for the mutagenicity of many PAHs (Hall & Grover, 1990). Later, the one-electron oxidation pathway (P450-peroxidase dependent), leading to reactive PAH radicals was discovered (Cavalieri & Rogan, 1992). More recently, the ortho-quinone pathway (al do-keto reductase [AKR] dependent) also resulting in reactive and redox active PAH o' quinones has been described (Penning et al., 1996).

In the radical cation pathway, PAH acts as a co-reductant of Fe^{3+} protopophyrin cation formed in the peroxidase cycle, yielding a radical cation at the most electron deficient carbon e.g. C6 of BaP.This reaction requires a peroxide co-substrate (Stadler & Lineback, 2009). Once formed the radical cation is short lived but can form depurinating DNA adducts leading to abasic sites. If unrepaired, these abasic sites can yield Guanine to Thymine (G-T)

transversion, a common mutation seen in tumor protoncogene (Puisiuex et al., 1991; Stadler & Lineback, 2009). In the diol epoxide pathway, the parent PAH undergoes an NADPH-dependent monoxygenation to yield an arene oxide e.g. 7R, 8S,-BaP oxide. This is then hydrated by epoxide hydrolase (EH) to yield (-) BaP 7, 8 dihydrodiol which can then undergo a further monoxygenation to yield the (+) anti BaP-7, 8 dihydrodiol epoxide (BPDE). There is evidence that this is the ultimate carcinogen. It forms a stable (+) anti BaPDE-N_2-dGuO adducts with naked and bulk DNA. These adducts can be detected in cell culture models and *in vivo* (Bentsen-Farmen et al, 1999; as cited in (Skupinska et al., (2004).

In the PAH o'quinone pathway, the intermediate transdihydrodiol (proximate carcinogen) for example (-) BaP 7, 8, dihydrodiol undergoes an $NADP^+$ dependent oxidation catalysed by the dehydrodiol dehydrogenase (DD) activity of AKRs (Smithgall et al., 1998). In humans, five isoforms have been implicated in this transformation; AKR1A1, AKR1C1 to AKR1C4. The reaction results in the formation of a ketol, which spontaneously reorganizes to form catechol. The catechol (o' hydroquinone) undergoes two sequential one electron oxidation steps in the presence of air to yield finally the o'quinone (Penning et al., 1996). As a result of these oxidation events, reactive oxygen species (ROs) are produced. Once formed the o'quinone is a reactive Michael acceptor that undergoes nucleophilic addition with bases in the DNA and RNA. In *vitro* PAH o'quinones can form both stable N_2-deoxyguanosine adducts and depurinating adducts (Puisiuex et al., 1991). It can also form conjugates with glutathione (catalysed by glutathione-S-transferase) and amino acid residue side chains in proteins. The conjugated PAH is not able to bind to DNA or protein. Hence glutathione has two functions; facilitation of excretion and protection of cells from mutation (Autrup et al, 1995; as cited in Skupinska et al., (2004). In the presence of cellular reducing equivalents, the o'quinones can be reduced back to the catechol for a subsequent round of auto-oxidation;

establishing a futile redox cycle that continues to generate ROs until the reducing equivalent is depleted (Skupinska et al., 2004).

It has been reported that a number of PAHs are activated by the bay region dihydrodiol epoxide pathway. The electrophilic products of this transformation; epoxydiols are hydrophilic. BaP for example, may be metabolized by cytochrome P-450. The metabolism is complex because the BaP particle has many reactive sites. Oxygen can be substituted in every site but not carbon 11, forming epoxides. Next in the reaction of hydrolysis those compounds could be transformed to trans-diols or can conjugate with glutathione. The occurrence of these reactions is limited by the epoxide's stability or enzyme access to the reaction site (Stadler & Lineback, 2009). The products of these reactions could be transformed to epoxides again as shown in the major routes for BaP shown in Figure 4.4.

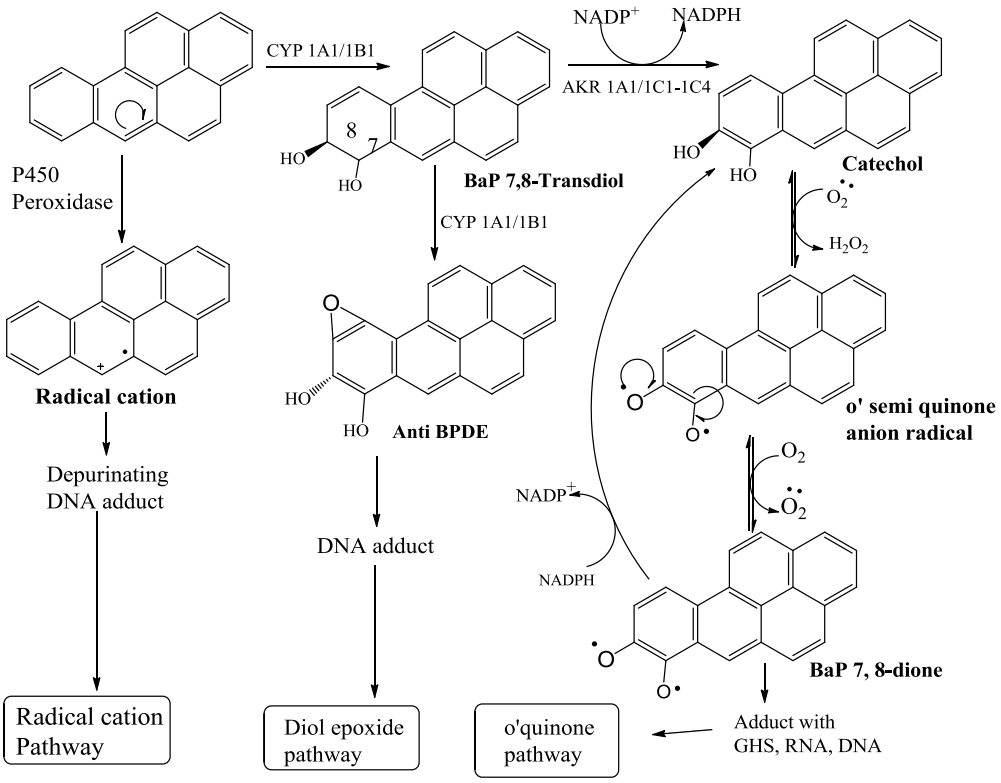


Figure 4. 4 Major routes for metabolic activation of BaP
From Stadler & Lineback,(2009)
Both the transformation of catechol to, and or semi quinone to quinone can cause oxidative damage to DNA.

4.5.3 Mode of action in carcinogenesis

The hydroxylation of PAHs causes an increase of activity towards DNA and proteins and the (electrophilic) species can bind to DNA rather than the excretory carrier molecules (Farombi, 2004). The products bind easily to the nucleophilic parts of the macro particles, forming adducts. Adduct formation can be a cause of protein damage or DNA mutation, which can lead to carcinogenesis (Skupinska et al., 2004).

BPDE is the most carcinogenic form of four possible isomers. Its half-life of 8 minutes is long enough to bind covalently to nucleophilic parts of proteins or DNA through the oxygen or nitrogen on purine (guanine and adenine). It is thought, the formation of adducts between the oxidized form of BaP with one of the organic bases e.g. guanine (Figure 4.5), can initiate carcinogenesis (Binkova et al., 2007). During the repair process the adducts are cut out from the strand and replaced by complementary bases. When the repairing system makes a mistake, the sequence of the nucleotides can be altered and in place of the damaged DNA a new helix with incorrect order of bases is created. In addition to base pair substitutions, PAHs may form bulky DNA adducts, resulting in frame shift mutations, deletions, strand breakage and a variety of chromosomal aberrations (Benford et al., 2009). Such a mutation can initiate carcinogenesis and further a neoplastic disease. In cancer prevention it is very important to stop the described processes at the earliest possible stage (Benford et al., 2009).

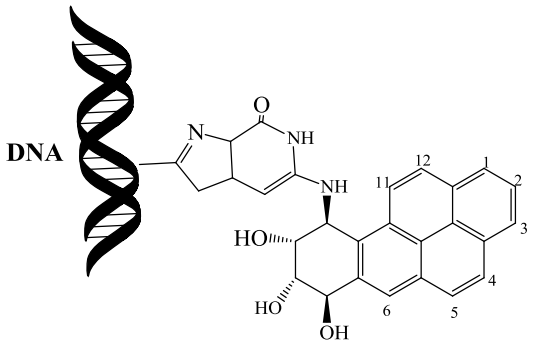


Figure 4. 5 BPDE-Guanine in DNA
A modification from Binkova et al.(2007)

PAHs are also known to initiate carcinogenesis or genotoxicity on exposure to ultraviolet radiations. When they absorb light energy, PAHs are excited to their upper energy states. The excited state energy can be lost by emitting light or heat, or transferred to molecular oxygen, solvent molecules or biological molecules in the cells to generate reactive, cell damaging intermediates (Yu, 2002). It has been observed that PAHs can cause DNA single strand cleavage and PAH-DNA adduct formation upon UV light irradiation. Generally PAHs are more toxic when exposed to simulated solar radiation than if they are kept in the dark and the increase in toxicity can exceed 100 folds. Thus the light activated PAHs can cause cellular damage and exert toxicity inducing carcinogenicity (Wang & Yu, 2005).

In Uganda, breast cancer is on the rise even among nonsmokers and is higher in young women. It is believed that carcinogenic, fat-soluble pollutants such as PAHs reach elevated concentrations in breast and other adipose tissues. This may ensure completion of the pathway for the initiation and promotion of carcinogenesis in the cells of those organs (http://www.canceractionny.org/breastcancerprevention).

4.5.4 Excretion

Following metabolism, hepatobiliary excretion and elimination through the faeces is the major route by which PAHs are removed from the body, independent of the route of administration. Urine is the other major excretory route, although it is quantitatively of minor importance compared to the bile, (Withey et al., 1991 as cited in WHO, 2000). The two major metabolites of BaP found in urine are 3-hydroxy and 9-hydroxybenzo[a]pyrene, part of them being conjugated to sulphate or glucoronic acid (Ramesh et al., 2001). The process of excretion of PAH compounds into the intestine, via the bile, reabsorption and return to the liver by the portal circulation has been demonstrated to occur for.DaA, BaP, Ant and Pyr (Ramesh et al., 2004). This enterohepatic recirculation extends the residence time of PAHs in the body and may lead to longer half-lives of PAH metabolites. This has however only been

studied in animal models with no confirmation in humans as yet (de Kok & van Maanen, 2000; as cited in EFSA, (2008).

4.5.5 Effects of PAHs on health

Many factors will determine whether if an individual is exposed to PAHs harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which the individual is exposed (breathing, eating, drinking, or skin contact), the other chemicals to which the subject is exposed, and the individual characteristics such as age, sex, nutritional status, family traits, lifestyle, and state of health (ATSDR, 1995b). PAHs have been reported to cause tumours in laboratory animals when they breathed these substances in the air, when they ate them, or when they had long periods of skin contact with them. Studies of people show that individuals exposed by breathing or skin contact for long periods to mixtures that contain PAHs and other compounds can also develop cancer (WHO, 1998).

Mice fed high levels of benzo[a]pyrene during pregnancy had difficulty reproducing and so did their offsprings. The offsprings of pregnant mice fed benzo[a]pyrene also showed other harmful effects, such as birth defects and decreased body weights. Similar effects could occur in people (WHO, 1998). Occupational exposures to high levels of pollutant mixtures containing PAHs have resulted in symptoms such as eye irritation, nausea, vomiting, diarrhoea and confusion. Mixtures of PAHs are known to cause skin effects in animals and humans such as irritation and inflammation. Anthracene, benzo(a) pyrene and naphthalene are skin irritants while anthracene and benzo(a) pyrene are reported skin sensitisers, (cause an allergic skin response) in animals and humans (Australia, 2009). Health effects from chronic or long-term exposures to PAHs may include cataracts, kidney and liver damage and jaundice. Naphthalene, a specific PAH, can cause the breakdown of red blood cells if inhaled or ingested in large amounts (Australia, 2009).

Animals exposed to high levels of some PAHs over long periods in laboratory studies have developed lung cancer from inhalation, stomach cancer from ingesting PAHs in food and skin cancer from skin contact (Albert, 1983). Long-term studies of workers exposed to mixtures of PAHs and other workplace chemicals have shown an increased risk of skin, lung, bladder and gastrointestinal cancers. Also reported are asthma-like symptoms, lung function abnormalities, chronic bronchitis and decreased immune function (WHO, 1998).

Results of epidemiological studies in coke oven workers, employees in aluminium production plants etc. indicate that the most significant health effect from inhalation exposure to PAHs is an excessive risk of lung cancer. The increase in lung cancer cases correlate closely with the time spent working on top of ovens where an average BaP concentration of about 30 mg m^{-3} has been detected (Albert, 1983; WHO, 1987). PAH-DNA adducts have also been implicated in the initiation of breast cancer in women (Sadikovic & Rodenhiser, 2006).

Further, in *vitro* studies showed that human breast epithelial tissue has the ability to metabolise PAHs to their ultimate mutagenic/carcinogenic moieties (Calaf & Russo, 1993). New findings from a Columbia University study showed that prenatal exposures to PAHs could reduce IQ in children at age three, increase behavioral problems at ages six and eight, and childhood asthma (Edwards et al., 2010; Perera et al., 2012). Genotoxic effects of some PAHs have been demonstrated in both rodents and *in vitro* tests using mammalian (including human) cell lines. Most PAHs are not genotoxic by themselves and must be metabolized to their diol epoxides, which then react with DNA to induce genotoxic damage. Genotoxicity plays an important role in the carcinogenicity process and may be in some forms of developmental toxicity as well (Schwerdtle et al., 2010).

PAHs undergo metabolic activation to diol-epoxides,which bind covalently to DNA. The DNA binding of activated PAHs is considered to be essential for the carcinogenic effect (Tarantini et al., 2011). DNA adducts have been found in various human tissues (Ziech et al.,

2011). In epidemiological studies, a positive correlation between the level of PAH exposure and the number of PAH-DNA adducts has been found, including that between coke oven exposure and PAH-DNA adducts in blood cells (Jedrychowski et al., 2013). If the adducts are left unrepaired, they may cause permanent mutations (Zhou et al., 2010). If these mutations are situated at critical sites, including tumour suppressor genes or oncogenes, they may lead to cellular transformation and the development of tumours. In some cases, specific mutations found in the Tp53 gene, the most commonly mutated gene in human cancers, are associated with exposure to certain carcinogens (Olivier et al., 2010). For example, the PAHs in cigarette smoke bind preferentially to the Tp53 gene sites called "hotspot" codons, where most smoking associated mutations are also found (Abedin et al., 2013). Such studies give support to the link between DNA adducts and the several cancer risks in humans.

Although only BaP has been adequately tested using dietary administration SCF proposed that PAHs should be regarded as potentially genotoxic and carcinogenic to humans. Because of the environmental ubiquity, prevalence in foods, their multiple routes of human exposure and the reported genotoxicity and carcinogenicity, the European Scientific Committee on Foods proposed that PAHs should be regarded as potentially genotoxic and carcinogenic to humans. The Committee recommended that, because of the non-threshold effects of these genotoxic substances, the levels of PAHs in foods, the environment and work places should be reduced to as low as is reasonably achievable (the ALARA principle) (FSAI, 2009).

The International Agency for Research on Cancer (IARC); The environmental protection agency (EPA) have classified PAHs based on their potential carcinogenicity to humans as shown in Table 4.1.

Table 4. 1 IARC classification of PAHs

Group 1	Group 2A	Group 2B	Group 3
Benzo(a)pyrene	Dibenzo(a,h)anthracene	Indeno(1,2,3-cd)pyrene	Benzo[ghi]perylene
		Chrysene	Phenanthrene
		Benzo(k)fluoranthene	Pyrene
		Benzo(b)fluoranthene	Fluorine
		Benzo(a)anthracene	Fluoranthene
		Naphthalene	Anthracene
			Acenaphthene

IARC (International Agency for Research on Cancer) classification: Group 1 = carcinogenic to humans, Group 2A = probably carcinogenic to humans, Group 2B = possibly carcinogenic to humans, Group 3 = not classifiable as to carcinogenicity to humans.

Chapter 5

VITAMIN E

5.0 Introduction

Vitamin E and other antioxidant components of the diet (vitamin C, carotenoids, selenium, flavonoids and several others) are at the forefront of the medical and nutritional sciences, because of significant advances in understanding of the relationship between oxidative stress in its various forms to the onset and/or control of many chronic diseases. These disease states including coronary thrombosis and cancer are focus points for consumer health interests and the medical community, hence dietary antioxidant components are highly recognized and valued by the consumer. Of the many such dietary components, vitamin E has attracted most interest because of its availability, strong marketing potential, overall health impact and central role in preventing oxidation at the cellular level (Galli et al., 2017).

5.1 Historical perspectives

Vitamin E was the fifth vitamin (fat-soluble) discovered in 1922 by Herbert McLean Evans and Katherine S. Bishop who found that a dietary deficiency in laboratory rats affected spermatogenesis in males and zygote retention in females. When fed wheat germs, the rats were able to reproduce. An alcohol substance of formula $C_{29}H_{50}O_2$ was later isolated from wheat germs in 1936. First named factor X and the antisterility factor, the vitamin was later designated vitamin E by Bishop, since its discovery closely followed the discovery of vitamin D. The name “tocopherol”, given to this compound by Evans research group was derived from the Greek words *tocos* (childbirth) and *phero* (to bring forth), and the chemical designation for an alcohol (*ol*) (Evans et al., 1936). Further research continued, and in 1936, Evans isolated alpha-tocopherol (Evans et al., 1936). Since then a variety of signs of vitamin E deficiency, such as muscular dystrophy, exudative diathesis, megaloblastosis, pulmonary

degeneration, nephrosis and liver necrosis, have been observed in experiments with several animal species (Creech et al., 1957).

Other notable events in the early history of vitamin E include the isolation of ß- and γ-tocopherol (ß-, γ-T) from vegetable oils in 1937 (Emerson et al., 1937), the determination of the structure of α-tocoperol in 1938 (Fernholz, 1938), synthesis of α- tocopherol in 1938 (Karrer et al., 1938), recognition of the antioxidant activity of the tocopherols in 1945 (Stern et al., 1947), recognition that α-tocopherol was the most effective tocopherol in the prevention of vitamin E deficiency (Emerson et al., 1937), isolation of δ-tocopherol (δ-T) from soybean oil in 1947 (Stern et al., 1947), identification of the four naturally occurring tocotrienols (α-T3, ß-T3, γ-T3, δ-T3) (Pennock et al.1964), documentation of naturally occurring tocopherols and tocotrienols in foods (Harris et al., 1950) and the proposal of vitamin E as an antioxidant against lipid peroxidation in 1968 (Mattill & Wolf, 2005).

Tocopherol fertility restoration assays provided the basis for quantification, so-called "International Units"(IUs). The acetate of [dl]-α– tocopherol was arbitrarily assigned the value of one IU per milligram. Natural α–tocopherol had an activity of 1.49 IU per mg, synthetic α-tocopherol an activity of one IU per mg; β, γ, and δ tocopherols had activities of 0.60 IU, 0.30 IU and 0.015 IU per mg, respectively (Best, 2001). The research data was used to set the initial 1968 recommended dietary allowance (RDA) for vitamin E at 30 IU. However, the United States Food and Nutrition Board now quotes Recommended Daily Allowances (RDAs) in milligrams rather than in IUs. For all adults over the age of 14 (male or female) the Board sets the RDA for natural RRR-α-tocopherol at 15 mg per day. There was also advice in the tenth edition of the RDA table (Food and Nutrition Board, 1989) only expressed as tocopherol equivalents (10 α-TE = 10 mg *d*-α-tocopherol = 15 IU). Further, research also pointed out that the vitamin E requirement increases with the intake of PUFA (Farwer et al., 1994)

5.2 Chemistry of vitamin E

5.2.1 Structure

Vitamin E is the collective term for fat soluble 6-hydroxychroman phenolic compounds that exhibit the biological activity of α- tocopherol measured by the rat resorption- gestation assay. Tocol, (2-methyl-2-(4',8',12'-trimethyltridecyl)-chroman-6-ol) (Figure 5.1), is the parent compound of the tocopherols (IUPAC-IUB (JCBN)., 1982).

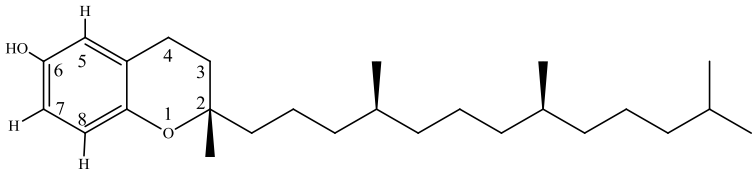


Figure 5. 1 The chemical structure of Tocol
From Zielińska & Nowak, (2014)

Natural vitamin E consists of α-, ß-, γ-, and δ-T and the corresponding α-, ß-, γ-, and δ-T3 (Figure 5.2). The tocopherols (Figure 5.2 (I)) are characterized by the tocol methylated to varying degrees at the 5, 7, and 8 positions. At position 2, there is a C16 saturated side chain. The tocotrienols (Figure 5.2 (II)) are unsaturated at the 3', 7', and 11' positions of the side chain. The specific tocopherols and tocotrienols (Table 5.1), therefore differ by the number and positions of the methyl groups on the 6- chromanol ring (Willson, 1983).

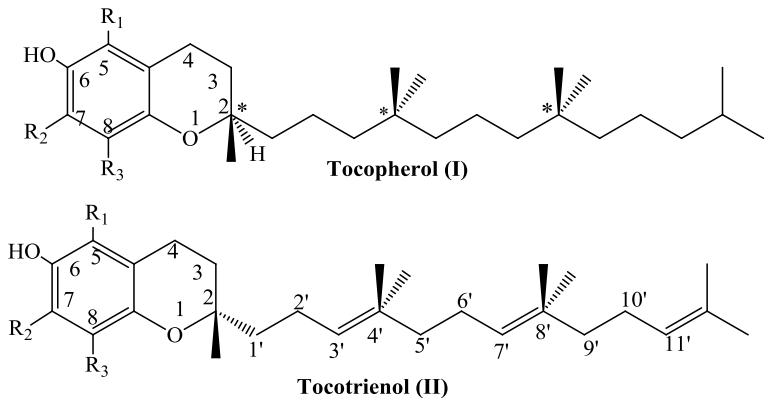


Figure 5. 2 The chemical structures of the tocopherols (I) and tocotrienols (II)
* Denotes a chiral centre.

Table 5. 1 Nomenclature and substituents in vitamin E

Vitamin E			Substituent at ring position		
Trivial name	Chemical name	Abbreviation	**R_1**	**R_2**	**R_3**
Tocopherols					
α-tocopherol	5,7,8-trimethyltocol	α-T	CH_3	CH_3	CH_3
β-tocopherol	5,8-dimethyltocol	β-T	CH_3	H	CH_3
γ-tocopherol	7,8-dimethyltocol	γ-T	H	CH_3	CH_3
δ-tocopherol	8-methyltocol	δ-T	H	H	CH_3
Tocotrienols					
α-tocotrienol	5,7,8-trimethyltocotrienol	α-T3	CH_3	CH_3	CH_3
β-tocotrienol	5,8-dimethyltocotrienol	β-T3	CH_3	H	CH_3
γ-tocotrienol	7,8-dimethyltocotrienol	γ-T3	H	CH_3	CH_3
δ-tocotrienol	8-methyltocotrienol	δ-T3	H	H	CH_3

Adopted from Bjrneboe, (1990)

5.2.2 Stereochemistry of vitamin E

The tocopherols possess three chiral centres at positions 2' 4' and 8', marked by * (Figure 5.1 (I)). Synthetic all- rac- α- tocopherol is a racemic mixture of equal parts of each stereoisomer. This means each tocopherol has 8 (2^3) possible optical isomers. The eight isomers all-rac- α- tocopherol are designated RRR, SRR, RSR, SSR, RRS, SRS, RSS and SSS (Figure 5.3). The alpha form of tocopherol (α-T) was originally designated d-alpha-tocopherol on the basis of its optical activity. According to that nomenclature therefore the all-rac- α-T was designated the dl α- T (Gagné et al., 2009)

Each form has slightly different biological activity but only the 2R-stereoisomeric forms (RRR-, RSR-, RRS-, and RSS) of α- tocopherol are considered active forms of vitamin E for humans (Food and Nutrition Board, 2000), with the most prominent being the RRR, (d)-α-tocopherol isomer form. It is what is commonly called "vitamin E" on nutrition/supplement

labels, and also the only form that can be referred to as the RDA for vitamin E. Alpha-tocopherols naturally occur in the RRR, (d)- isomer form, which is more active than the synthetic racemic dl- isomer form (Gagné et al., 2009).

The tocotrienols arising from 2-methyl-2-(4',8',12'-trimethyltrideca-3',7',11'-trienyl) chroman-6-ol (nonmethylated ring structure) have only one chiral center at position 2 (Figure 5.2 (II)). Consequently, only 2R and 2S stereoisomers are possible. Unsaturation at positions 3' and 7' of the phytyl side chain permits four cis/trans geometric isomers. Only the 2R, 3'-trans, 7'-trans isomer exists in nature (Mayer et al., 1967; Whittle et al., 1966). The α-tocopherol form constitutes 90 percent of the tocopherols found in humans, with the largest quantities in blood and tissues (Chow, 1975).

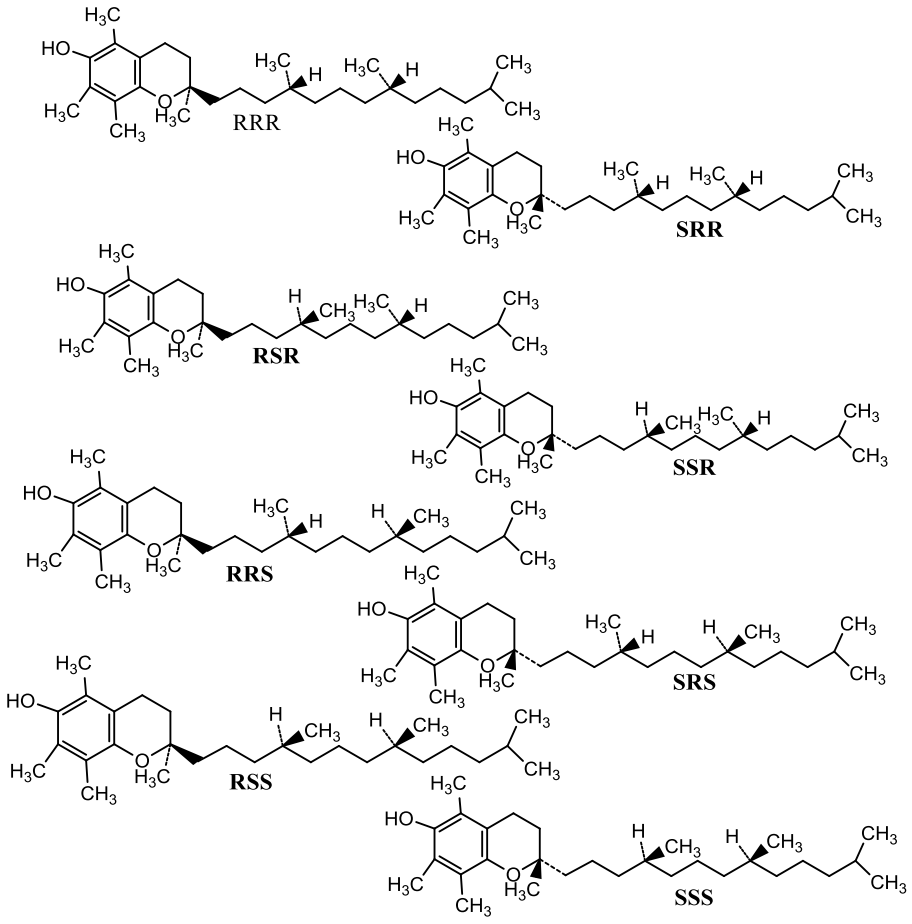


Table 5. 2 Stereoisomers of α-tocopherol
Adapted from Traber, (1999)

Normal blood plasma consists of 83-percent d-α-tocopherol and 13-percent γ-tocopherol. The preferential distribution of α- tocopherol in humans over the other forms of tocopherol stems from the faster metabolism of the other forms and from the α-tocopherol transfer protein (α-TTP). It is due to the binding affinity of α-tocopherol with α-TTP that most of the absorbed β-, γ- and δ-tocopherols are secreted into the bile and excreted in the faeces, while α-tocopherol is largely excreted in the urine (Schmölz et al., 2016). The α-tocopherol form also accumulates in the non-hepatic tissues, particularly at sites where free radical production is greatest, such as in the membranes of the mitochondria and endoplasmic reticulum in the heart and lungs (Rizvi et al., 2014). It has been found that long-term supplementation with just a d-alpha-tocopherol vitamin E supplement results in blood plasma levels of d-gamma-tocopherols being lowered by 30 to 50 percent. As a result, some researchers now recommend, to those who are interested in taking a vitamin E supplement, to select one with mixed tocopherols (Wu et al., 2007).

5. 3 Sources and forms of vitamin E

Because only plants can synthesize vitamin E, animals must get their vitamin E by eating plants or by eating animals that have eaten plants.Vitamin E and other mixed tocopherols are isolated from vegetable oil distillates (VODs) and concentrated to contain d-alpha, d-beta, d-gamma and d-delta tocopherols. Foods high in vitamin E (with their Daily values; DV), include sunflower seeds (7.4 mg; 49%), almonds (7.3 mg; 49%), avocados, (4.2 mg; 28%), spinach (3.7 mg; 25%), butternut squash (2.6 mg; 18%), kiwifruit (2.6 mg; 18%), broccoli (2.3 mg; 15%), trout (2 mg; 13%), olive oil (1.9 mg; 13%), shrimp (1.9 mg; 12%). The current Daily Value (DV) for vitamin E is 15 mg (USDA, 2018b). Vitamin E also occurs ubiquitously in cereal grains such as wheat, rice and barley, and in certain vegetable oils like palm oil or bran oil but also in meat and eggs (Wojcik et al., 2010).They naturally protect fats

and oils from oxidation. Commercial available sources of vitamin E can be classified into the following distinct categories:

- *Natural Vitamin E:* this is what most people refer to as vitamin E; it is the non-esterified form called d-alpha-tocopherol, (RRR alpha tocopherol); an alcohol that occurs in nature as a single stereoisomer. These come from vegetable (primarily soy) and sunflower oil (Machlin, 1991).
- *Semi-Synthetic, Esters:* manufacturers commonly convert the phenol form of the vitamin (with a free hydroxyl group) to esters, using acetic or succinic acid to form the d-alpha tocopheryl acetate or the d-alpha tocopheryl succinate respectively.These tocopheryl esters are more stable (esters are less susceptible to oxidation) during storage because they are not acting as antioxidants in their esterified form. The ester forms are de-esterified in the gut (by the enzyme esterase) and then absorbed as the free tocopherol. Several studies indicate the rate of absorption of these forms of tocopheryl esters and free tocopherols have similar bioavailability.
- *Synthetic Vitamin E:* the synthetic form of vitamin E, dl-alpha-tocopherol is made by coupling trimethyl hydroquinone (a reduced benzoquinone) with isophytol (acyclic terpenoid). Synthetic vitamin E is racemic mixture containing all the eight isomers of alpha-tocopherol (all racemic) in approximately equal amounts, so it has approximately half of the biological activity of natural vitamin E.
- *Fractionated forms of vitamin E*:the most-common fractionated forms are: natural mixed tocopherols and high d-gamma-tocopherol.

5.4 Biological roles of Vitamin E

Vitamin E is an interesting group of compounds, able to exert many and different biological activities in plant, animal and human cells. α-tocopherol has the greatest biological

activity when compared to other forms of vitamin E, because it has higher absorption, higher deposition in tissues and low fecal excretion (Cortinas et al., 2005).

5.4.1 Vitamin E (α-T),as an antioxidant

Vitamin E functions with other lipid- and water-soluble antioxidants to provide living systems an efficient defense against free radicals and the damage that they impart at the cellular level. Free radicals are chemical species capable of independent existence that contain one or more unpaired electrons. According to Bramley et al., (2000), free radicals are normally generated in living tissues in some of the following ways: 1) In the mitochondria through production of superoxide and hydrogen peroxide (H_2O_2) by normal respiration; 2) By phagocytes leading to production of hydrogen peroxide (H_2O_2), nitric oxide (NO·), and hypochlorite (ClO^-) in association with the respiratory burst; 3) Peroxisomes causing degradation of various substances including fatty acids to yield hydrogen peroxide (H_2O_2); 4) Cytochrome P-450 enzyme catalysis of various oxidation reactions; 5) Low-wavelength irradiation that generates hydroxy radicals (OH·) from water and 6) Ultraviolet irradiation causing cleavage of the O-O covalent bond in H_2O_2 to produce two OH· radicals.

These reactive oxygen species (ROS) together with reactive nitrogen species (RNS) are the primary prooxidants that induce oxidative stress in living systems or initiate autoxidative events in raw and processed foods. Reactive nitrogen species, particularly nitric oxide (NO·), acts as a biological messenger with regulatory functions in the central nervous, cardiovascular, and immune systems (del Rio et al., 2002).

Since generation of free radicals occurs in hydrophilic and hydrophobic locations, both water- and lipid soluble antioxidants are required to limit free radical damage. α-Tocopherol is the primary lipid-soluble antioxidant in mammalian and plant cells located in the cell membranes and available to protect lipoproteins (Traber & Atkinson, 2007). It functions as a primary, chain-breaking antioxidant, scavenging peroxy free radicals. Lipid generated free radicals

have greater affinity for reaction with α-T than with PUFA located in membrane phospholipids. This facilitates the protection of PUFAs. α-Tocopherol acts as an efficient chain-breaking antioxidant by rapidly transferring its phenolic H^+ to lipid peroxyradicals, while itself becoming a relatively inactive low energy free radical—the α-tocopheroxyl radical (Figure 5.3), which is resonance stabilized as shown in Figure 5.4 (Decker, 2002)

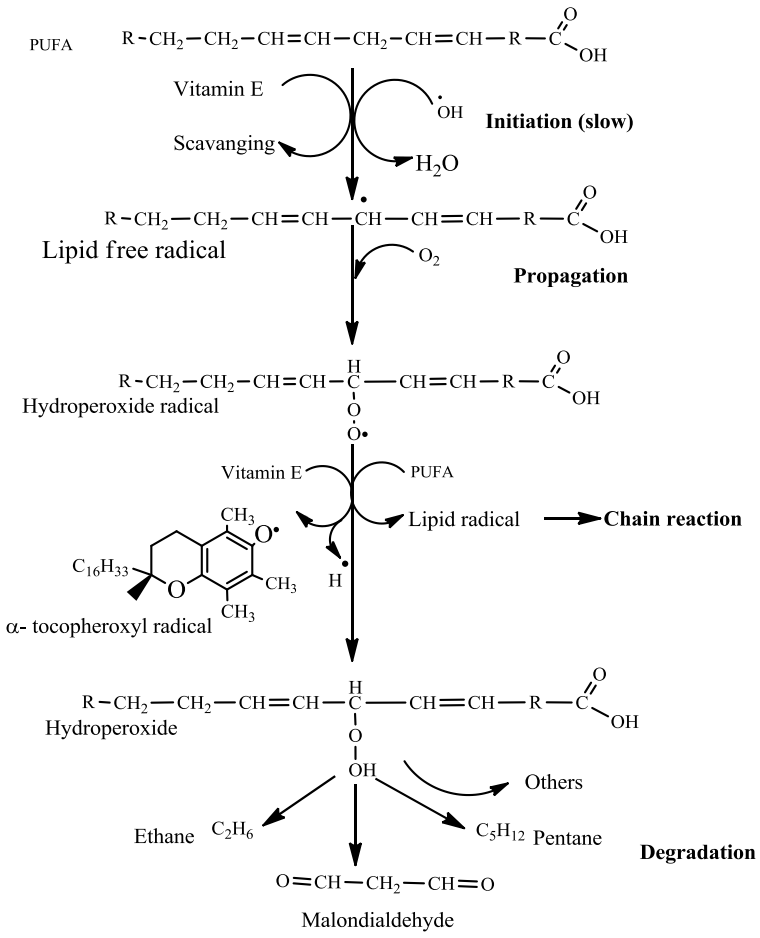


Figure 5. 3 Lipid peroxidation and vitamin E scavanging of free radicals
Adopted from Bjrneboe, (1990)

In the membrane α- Tocopherol acts both as a membrane stabilizing component as well as an antioxidant. Additionally, alpha-tocopherol also protects the fats in low-density lipoproteins (LDLs) from oxidation. Oxidized LDLs have been implicated in the development of cardiovascular disease (CVD). Several studies have established vitamin E supplementation as a way to help prevent or treat various chronic disease states, including:

aging, arthritis, cancer, CVD, cataracts, dementia (impaired cognitive function), immune function, platelet hyper-aggregation (reduction), prostaglandin production (reduction) and reproduction. Commercially, vitamin E is also used as an antioxidant to preserve many (bulk and finished product) PUFAs and oils from oxidation (Katalin et al., 1996)

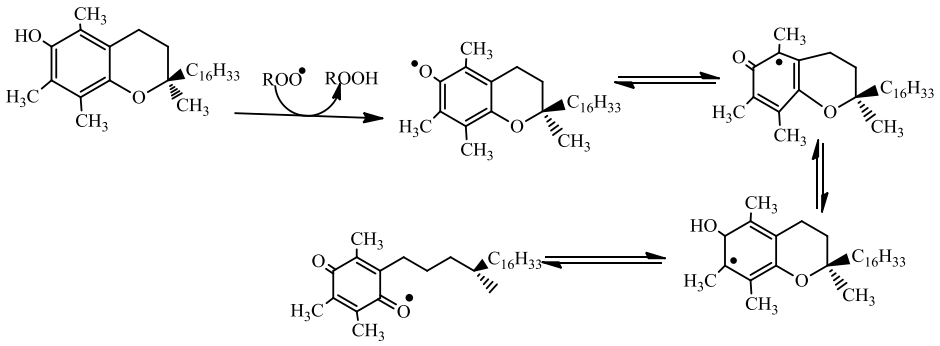


Figure 5. 4 Resonance stabilization of α-tocopheroxyl radical
Adapted from (Decker,(2002)

As far as biological roles are concerned, vitamin E derivatives have three main distinct domains, described as: (a) the functional domain, responsible for the antioxidant activity and, therefore, Vitamin E properties, epitomized by the hydroxyl group in α-tocopherol; (b) the signalling domain, comprised of the aromatic rings (phenol-, chromanol-) and activated by the monoesterification of dicarboxylic acids with the phenol oxygen; and (c) the hydrophobic domain, responsible for docking the agents in circulating lipoproteins and biological membranes (Augustyniak et al., 2010).

The animal body also has some intrinsic antioxidant defense systems in addition to vitamin E; divided into primary and secondary defense systems. The primary defense is composed of three important enzymes that prevent the formation of or neutralize free radicals: (1) glutathione peroxidase, which donates two electrons to reduce peroxides by forming selenoles and also eliminates peroxides as potential substrates for the Fenton reaction; (2) catalase, that converts hydrogen peroxide into water and molecular oxygen and has one of the biggest turnover rates known to man, allowing just one molecule of catalase to convert 6

billion molecules of hydrogen peroxide; (3) superoxide dismutase converts superoxide anions into hydrogen peroxide as a subtrate for catalase (Rahman, 2007). The secondary enzymatic defenses include (1) glutathione reductase, with an important role in protecting haemoglobin, red cell enzymes, and biological cell membranes against oxidative damage by increasing the level of reduced glutathione in the process of aerobic glycolysis, and (2) glucose-6-phosphate dehydrogenase, whose activity is important for cell growth (Tian et al., 1998).

In addition to the natural antioxidants (enzymatic and non enzymatic) currently in use, there also exist synthetic antioxidants. These include butylated hydroxytoluene (BHT); butylated hydroxyanisole (BHA); tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG). Research has shown that the antioxidative capacities of these synthetic antioxidants vary in the order: BHT > PG > BHA > TBHQ (Marmesat et al., 2010). However, whereas synthetic antioxidants could provide cheaper options, they have been reported to be dangerous for human health. Thus, natural antioxidants continue to be ideal as food additives, not only for their free radical scavenging properties, but also because they are healthier and safer than synthetic products (Tavasalkar et al., 2012).

5.4.2 Other functions of vitamin E

Vitamin E has a wide range of other functions in the body; apart from anioxidative role, it is primarily crucial for fertility in humans and livestock species (Surai et al., 2006). Researches have documented the benefits of Vitamin E on improving reproductive traits in male poultry (Cerolini et al., 2006; Li et al., 2009). A study by Alm El-Dein et al., (2013) showed that vitamin E constitutes 88% of chicken semen antioxidant capacity and that a high level of vitamine E in the diet improves the semen physical characteristics and also the normal hatchability of eggs (Amiri Andi et al., 2006).

Vitamin E has also been shown to play a role in the normal development and function of the immune system. The immunomodulatory effects of vitamin E have been reported in

humans and a variety of animal species, and were most evident in very young, very old, and immunocompromised individuals. As Gore & Qureshi, (1997) reported, there was enhanced cell-mediated and humoral immunity in broiler chicks receiving 10 IU VE *in ovo* on day 18 of incubation. Further, reports indicate that vitamin E improves immunity to *Escherichia coli* infection, coccidiosis, and infectious bursal and Newcastle diseases. Dietary vitamin E supplementation in the form of α-tocopherol acetate, significantly affects T cell differentiation in the thymus and alters the proportions among T cell subsets in the thymus and spleen of broiler chickens (Erf et al., 1998).

Researchers have also reported on the role of vitamin E in the blood cloting system by inhibiting platelet integration, and in providing protection against the toxicity of heavy metals; cadmium, mercury, arsenic, selenium and lead (Bouts & Gasthuys, 2003). In addition, α-tocopherol has been known to inhibit protein kinase C, 5-lipoxygenase and phospholipase A2, and to activate protein phosphatase 2A and diacylglycerol kinase (Gagné et al., 2009).

5.5 Pharmacokinetics of Vitamin E

Poultry cannot synthesize vitamin E, therefore, vitamin E requirements must be given from dietary sources (Ziaei et al., 2013). The dietary recommendations of vitamin E for poultry species vary among species. Poultry feed can be supplemented with 10 IU of vitamin E per kg feed (1 IU = 0.67 mg *dl*-α-tocopheryl acetate) for chickens aged up to six weeks, 5 IU/kg feed for chickens aged over six weeks, 12 IU/kg feed for turkeys aged up to eight weeks, and 10 IU/kg feed for turkeys aged over eight weeks. For ducks and Japanese quail, feed can be supplemented with 10 IU/kg feed and 12 IU/kg feed, respectively, for starting and growing birds (Rengaraj & Hong, 2015). The vitamin E therefore has to be absorbed and distributed to the tissues where its functions are needed and the residue excreted.

5.5.1 Absorption and Distribution

Vitamin E absorption is low in humans. Being a lipid, absorption from the intestinal lumen is dependent upon biliary and pancreatic secretions, micelle formation, uptake into enterocytes, and chylomicron secretion, and then into circulation via the lymphatic system. Absorption occurs in the median portion of the small intestine from the intestinal lumen. All forms of vitamin E (solubilized, natural and synthetic) have similar intestinal absorption (IOM, 2000). Absorption of vitamin E is closely related to fat absorption which is facilitated by the lipase enzymes of the pancreas and bile. Moreover, vitamin E is normally absorbed as an alcohol so, if the supplement contains the ester form, this should first be hydrolyzed before absorption (Bouts & Gasthuys, 2003). A study by Ziaei et al., (2013) indicates that in chickens, absorption of vitamin E is impaired by severe selenium deficiency. Selenium alleviates vitamin E deficiencies by permitting higher levels of vitamin E to be absorbed.

Vitamin E is fat soluble and is transported in the blood by the plasma lipoproteins and erythrocytes. It is distributed throughout the body and is primarily stored in adipose (fat) tissues and various organs. The human body stores about 40 mg/kg and 77 percent is stored in adipose tissue. It is transported to the liver, packaged into very low density lipoproteins (VLDLs) and excreted back into the circulation. After its intestinal absorption vitamin E is packaged into chylomicrons, which along the lymphatic pathway are secreted into the systemic circulation. By the action of lipoprotein lipase (LPL), parts of the tocopherols transported in chylomicrons are taken up by extrahepatic tissues, and the remnant chylomicrons transport the remaining tocopherols to the liver. Here, by the action of α-TTP, a major proportion of alpha-tocopherol is incorporated into nascent very low density lipoproteins (VLDLs). Besides the LPL action, the delivery of α-tocopherol to tissues takes place by the uptake of lipoproteins by different tissues throughout their corresponding receptors (Herrera & Barbas, 2001).

The distribution of vitamin E isoforms varies from tissue to tissue. A study by Packer et al., (2001) on mice showed that, in individuals that were fed a diet not specifically enriched with tocotrienols, up to 15% of total vitamin E was composed of tocotrienols; the brain contained no detectable α-tocotrienol levels; in other tissues, 99% of the vitamin E was present as α or γ-tocopherol. These observations indicate that tissues may possess the ability to regulate the vitamin E composition individually. Small amounts of vitamin E will persist for a longer time in the body but the major stores will be exhausted rapidly due to polyunsaturated fatty acids in tissues (Bouts & Gasthuys, 2003).

5.5.2 Metabolism

Alpha-tocopherol is oxidized to the tocopheroxyl radical that can be reduced back to the un-oxidized form by reducing agents such as vitamin C. Further oxidation of the α-tocopheroxyl forms tocopheryl quinone. The tocopheryl quinone (Figure 5.5), is not converted back to tocopherol and is eventually excreted. Tocopherols are extremely resistant to heat but readily oxidized. Natural vitamin E is subject to destruction by oxidation, which is accelerated by heat, moisture, rancid fat, copper, and iron. Alpha-tocopherol is an excellent naturally occurring antioxidant that protects carotene and other oxidizable materials in the feed and in the body. However, in the process of acting as an anti-oxidant, it is oxidized and becomes biologically inactive (Sitrin & Bengoa, 1987).

5.5.3 Excretion

Before excretion vitamin E is extensively metabolized. Previous studies indicated that two major urinary metabolites of α-tocopherol, tocopheronic acid and the tocopheronolactone derived therefrom were formed. Both metabolites are excreted in the urine as glucuronides or sulfates (so-called Simon metabolites) (Brigelius-Flohé et al., 2002). Compared to α-T, these compounds have an open hydroxychroman ring and a shortened side chain. Measurement of Simon metabolites in urine may therefore represent a useful, non-invasive marker that reflects

the extent to which α-T has reacted as a radical scavenger in vivo. However, the existence of these metabolites has been questioned, with some evidence suggesting that they are formed only as artefacts during Vitamin E is excreted mainly via bile, urine, faeces and the skin. But the major route of excretion of ingested vitamin E is fecal elimination. Vitamin E metabolites appear to be primarily eliminated via the kidneys. This occurs when the vitamin is oxidized to hydroquinone which is conjugated to form glucoronate. The glucoronate is then excreted into bile or further degraded in the kidneys and excreted in the urine (Moore & Ingold, 1997).

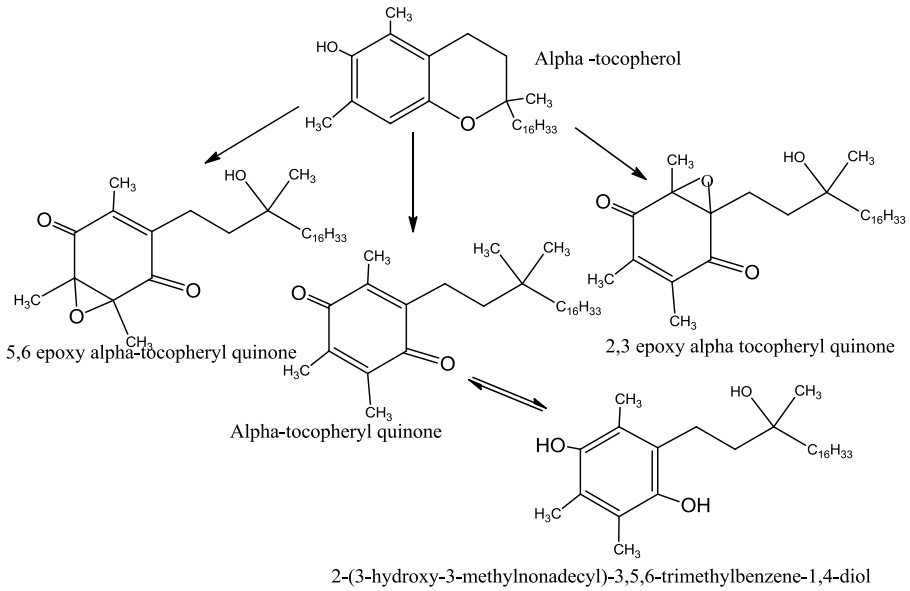


Figure 5. 5 The oxidation of α-tocopherol
From Yamauchi, (2003)

Measurement of Simon metabolites in urine may therefore represent a useful, non-invasive marker that reflects the extent to which α-T has reacted as a radical scavenger *in vivo*. However, the existence of these metabolites has been questioned, with some evidence suggesting that they are formed only as artefacts during sample preparation. As urinary α-T metabolites are thought to be excreted as conjugates of glucuronides or sulfates, urine samples have to be hydrolysed chemically or enzymatically prior to their analysis (Wu & Croft, 2007).

5.5.4 Deficiency in Poultry

Vitamin E has been shown to be essential for integrity and optimum function of reproductive, muscular, circulatory, nervous, and immune systems. Vitamin E is stored throughout all body tissues, with highest storage in the liver. Vitamin E is an essential nutrient for chickens of all ages, and its deficiency causes several disorders such as:

Encephalomalacia: a serious disorder that causes permanent tissue damage to the chicken's brain, as a result of localized softening of the cerebral. This form of vitamin E deficiency occurs most often in chickens that are getting fed a diet containing high levels of polyunsaturated fatty acids of the linoleic acid series (such as that found in many cooking oils) and low levels of vitamin E. Dilauryl succinate has also been documented to induce encephalomalacia in chickens (Pappeniieimear & Goerncir, 1931).

Exudative diathesis (ED): due to vitamin E deficiency first occurred in chicks on experimental diets used to study vitamin K (Dam & Glavindi, 1938). It was characterized by massive accumulations of exuded plasma in the subcutis and skeletal muscle, with petechiation scattered throughout the affected areas. It has since occurred on a variety of diets deficient in vitamin E and has been delayed or prevented by the addition of selenium and of alpha-tocopherol to the deficient diet. Clinical signs observed include greenish-blue discolouration of the skin in localized areas of the chick's body, along with edema and hemorrhages, often resulting in bowlegged posture and pendulous (loosely hanging) crop in the throat latch area (Sciiwartz, 1957).

Nutritional muscular dystrophy: also known as white muscle disease or nutritional myopathy, is a disease that primarily affects the chicken's striated muscles. It involves progressive weakness and degeneration of the muscles that control movement. Affected chicks are often unable to stand or walk, and are seen on the ground with their legs spread laterally. It has proved to be a complex deficiency involving selenium and sulphur amino

acids as well as excess dietary linoleic acid (Calvert et al., 1964). It has occurred on diets containing levels of antioxidants which have prevented encephalomalacia. Addition of linoleic acid to the diet has increased proportionately the incidence and severity of muscle lesions and increased the requirement of vitamin E for their prevention (Calvert et al., 1964).

PART 2: RESEARCH WORK

Chapter 6

INTRODUCTION

With the rising production and consumption of food of animal origin in the current decade, meat quality and food safety have become major concerns for all those along the production chain. Consumers typically consider colour, flavour/smell and texture as the most immediate signs of meat quality. These properties are determined by the oxidative stability of the meat that depends among others, on the balance between antioxidant and pro-oxidant components in muscles (Descalzo & Sancho, 2008). The PUFAs in the cell membranes, metal ions, haem proteins and reactive oxygen species are the major pro-oxidant components. On the other hand, there are reported endogenous antioxidant defenses like superoxide dismutase (SOD); glutathione peroxidase (GPX); glutathione reductase, catalase, Vitamins E and C, and β-carotene within the animals, and exogenous antioxidant molecules such as carotenoids, polyphenols, vitamin E and ω-3 of dietary origin, that are able to extend meat shelf life by counteracting the lipid oxidative reactions (Morrissey et al., 1998).

Poultry meat consumption has increased tremendously over the past decades and chicken meat alone constitutes over 30% of the global meat consumption (Belova et al., 2012). This is associated with the meat's low fat and high essential fatty acid (PUFA) contents, rise in income per capita, urbanization, general acceptability of poultry meat by world cultures and religions and relatively lower costs of both production and consumption. Chicken meat is normally eaten grilled, roasted, fried, broiled, toasted or barbecued. These alimentary processes help destroy harmful microorganisms, increase digestibility of proteins and raise organoleptic profiles; but may also lead to formation of carcinogenic substances principally, polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs), both of which have been implicated in carcinogenesis, both in laboratory animals and in humans

(Kizi et al., 2011; Lynnette, 2010). The formation of these carcinogens is reported to depend on the temperature, fat content, cooking method, the duration and lipid oxidation among others; being generally higher at elevated temperatures. Moreover, many consumers prefer well-done, strongly heated meat and meat products that could therefore be heavily laden with these carcinogenic compounds, thus lowering food safety (Kizi et al., 2011).

PAHs are ubiquitous environmental contaminants mainly formed by the incomplete combustion of organic matter, through the Maillard reaction that employs free radical mechanisms at some stages (Agerstad & Skog, 2005). Of the several PAH compounds known, sixteen are considered to be priority pollutants (US EPA, 1970). Among them, benzo (a) pyrene, the most widely studied carcinogenic PAH, is used as a marker for the rest of the PAH compounds found in foods and the environment (Scientific Committee on Food, 2002).

Vitamin E has been singled out as the principal natural antioxidant, which can protect lipid molecules and cell membranes against oxidative damage (Castellini et al., 1998). The chain breaking antioxidant function of vitamin E is known to stop the self-perpetrating production of lipid peroxides that leads to food spoilage (Benzie, 1996). Vitamin E has also been recognized as an essential nutrient for health and growth of all species of animals. These roles of vitamin E are due to its involvement in nutritional myopathy, immune responsiveness and prostaglandin biosynthesis (Adebiyi et al., 2011).

Studies have also reported inhibitory effects of vitamin E (an antioxidant) on formation of carcinogenic compounds during meat processing (Liao et al., 2009; Sugimura et al., 2004). A similar inhibitory effect has been observed in cooked beef when vitamin E is added during cooking (Wong et al., 2012). Previous studies have shown that dietary vitamin E has numerous positive effects on beef quality, such as colour and lipid stability (Descalzo et al., 2007; Juárez et al., 2011). However to date no studies have investigated the role of endogenous tissue levels of vitamin E on the formation of PAHs in poultry meat. Further, for

optimum efficacy in preventing lipid oxidation and improving health, growth, and for inhibiting the formation of PAHs, the level of vitamin E in the organism needs to be higher than usual for the excess to be deposited in the cellular membranes. This can be met through supplementation in the feeds or through intramuscular injection. When added to meat post mortem, the vitamin will not be physiologically and naturally incorporated into cellular membranes (Liu et al., 1995). Moreover, the intramuscular injection of vitamin E is preferred because reports say it reduces on the loss of vitamin E activity in feedstuff, and ensures a more standardized administration in the animals (Maiorano et al., 2015)

This study therefore aimed at assessing the effect of intramuscular vitamin E injection on growth performance, meat quality and on formation and mutagenic activity of processed meat of Cobb 500 broilers reared under tropical conditions.

The specific objectives of the research, carried out on Cobb 500 broiler chickens raised under tropical conditions, were to assess the effect of intramuscular vitamin E injection on:

1. growth performance;
2. oxidative stability of meat;
3. countering the formation of polycyclic aromatic hydrocarbons during grilling of meat.

Chapter 7

MATERIALS AND METHODS

7.1 Sampling and sample treatment

This study was carried out from September 2017 to September 2018 in Kampala (Uganda); samples being collected from Gulu Municipality which stretches from about $2^{o}46'54.0$ N to $32^{o}17'57.0$'E. Gulu municipality is 340 km (210 miles) by road from Kampala, Uganda's largest and capital city. A survey of prominent poultry farms was carried out in the peri-urban areas of the municipality. A small poultry farm (~300 birds per farm) was identified at Aywee sub-ward, Pece Division, choice of the farm being influenced by ease of accessibility, safety and relatively lower costs of both purchase and rearing of the birds.

Thirty, 28d old Cobb 500 chicks were bought and randomly assigned to three treatment subgroups (n = 10, 5 replications per experimental group), which were intramuscularly injected on left pectoral muscle (Figure 7.1) with 0.25 mL (12.5 IU), (T1) and 0.5 mL (25 IU), (T2) of *dl*–α–tocopheryl acetate (50 mg/ mL) (Vitalene® E, Fatro, Bologna) and 0.5 mL of physiological saline (C). The birds were raised in pens fitted with the solid floor with free access to the outdoor paddock and were confined to indoor pens during night. Chickens were fed *ad libitum* with the same diet and had free access to water.

Figure 7. 1 Intramuscular vitamin E administration

7.2 Slaughter surveys

At 42 days of age, all birds were individually weighed (after a fasting period of 12 h), and slaughtered. The carcass weights were measured after removing the blood, feathers, skin, head, feet, and all internal organs (evisceration). Carcass yields were calculated as percentages of the final live weights after fasting. The major cuts including breast muscles, leg muscles (thigh and drumsticks), wings and backs + necks were similarly weighed and their yields computed as percentages of the eviscerated carcass weights.

7.3 Meat quality traits

After slaughter, the left and right pectoral muscles (Figure 7.2) were packed in a cooler box and transported frozen to the Department of Food Science, Nutrition and Biotechnology, College of Agriculture and Environment, Makerere University- Uganda for further analyses.

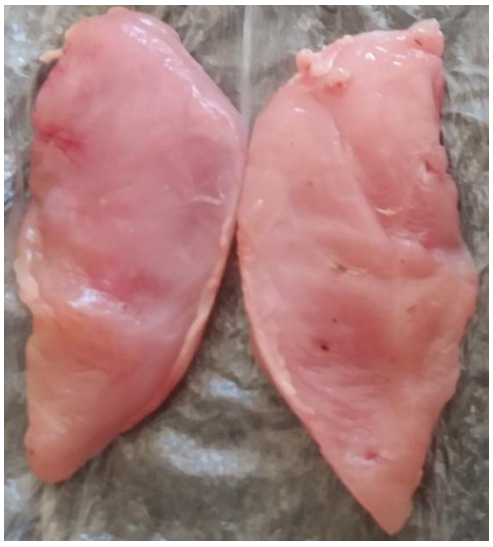


Figure 7. 2 Right and left pectoral muscles of a Cobb 500 broiler chicken

7.3.1 Determination of pH

The ultimate pH (pHu) was determined 24 hours *post mortem*, on the right pectoral muscle using a portable, 68 X 247809 2/05 Rev 3 Oakton Instruments – Malaysia pH tester, equipped with a penetrating glass electrode.

7.3.2 Determination of Water holding capacity

WHC, expressed as expressible juice, was measured on the right pectoral muscle 24 h *post morterm* after chilling; using the filter paper press method described by Grau & Hamm, (1953) and modified by Trout (1988). A cubical piece of meat (0.3 g) was placed in between two Whatmann No 1 filter papers (diameter 55 mm) and a 1 kg mass applied for 5 minutes (Figure 7.3).

The water squeezed out was absorbed by the filter paper and was related to the amount of "loose" water in the sample. The amount of water released was then measured indirectly as the area of the ring of expressed juice using a planimeter. From the area determined, the water holding capacity was computed.

Figure 7. 3 The filter paper press method determination of WHC

7.3.3 Determination of total lipids

Total lipids were determined by the method of Folch et al., (1957) as modified by Bligh & Dyer, (1959). A piece of the pectoral muscle (10 g) was weighed out and chopped into small pieces. The pieces were transferred into a 500 mL Pyrex bottle and mixed with methanol/chloroform mixture (1:1, 200 mL). The mixture was homogenized with an ultra-turax (Ika® Ultraturax T25) homogenizer for about 2 minutes. The bottle was closed and kept in an oven at 60^{o}C for 20 minutes, removed and cooled under water to ambient temperature.

Chloroform (100 mL) was added, the mixture shaken and filtered through the Buchner funnel with a Whatman No 1 paper.

The filtrate was transferred into a 500 mL bottle and mixed with aqueous potassium chloride (1 M, 100 mL). The mixture was shaken for 1 minute and kept over night in a refrigerator at 4^{o}C, transferred into a 250 mL separating funnel arranged under the hood and the lower layer collected into a dry 250 mL bottle. Anhydrous sodium sulphate (2 spatula end fulls) were added and the mixture shaken for about 3 minutes and kept at 4^{o}C for 2 hours. Labeled round bottomed flasks were dried in an oven set at 105^{o}C for 40 minutes, cooled under the dessicator, weighed (*Pi*) and used to collect the filtrate from the bottle. The filtrate was evaporated to dryness under a rotary evaporator set at 40^{o}C. The flasks were cooled under the dessicator and weighed again (*Pf*). The percentage of the extracted total lipids in the samples were then calculated using the formula:

$$TEL = \frac{P_f - P_i}{P_c} \times 100$$

Where *Pc* = mass of the sample used. Determinations were done in duplicate.

7.3.4 Measurement of oxidative stability

For oxidative stability evaluation, breast muscle samples were analyzed as raw meat after storage at 4°C for 24 hours (the meat being previously stored in the freezer at -20^{o}C). Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) assay methods (Sorensen & Jorgensen, 1996) with modifications (Zeb & Ullah, 2016). Briefly, the right pectoral muscle (5.0 g) raw meat was homogenized with an ultra turrax (Ika® Ultraturax T25) homogenizer in a 50 mL Falcon tube containing the extraction solution (a mixture of 7.5% trichloroacetic acid, 0.1% propyl gallate, 0.1% EDTA, 15 mL) and filtered.

The filtrate (1.0 mL) was transferred into a 10 mL Pyrex glass tube with Teflon lined cap; containing TBA solution (0.02 M, 1.0 mL). Blanks were prepared by mixing the

extraction solution (1 mL) with the TBA solution (0.02 M, 10 mL); both the blanks and extract mixtures were kept in an oven at 100°C for 40 minutes. The set up was then removed and the tubes cooled under water (10 minutes), to ambient temperature. Absorbances were measured at 532 nm with a correction for turbidity at 600 nm, in single use Kartell cuvettes - Italy. The TBARS values were determined using a standard curve prepared from 1, 1, 3, 3-tetraethoxy-propane (TEP) (Sigma Aldrich), from standard solutions (0, 1, 2, 4, 5 μM), and expressed as mg of malondialdehyde (MDA) per kilogram of sample. Analyses were performed in duplicate.

7.3.5 Determination of Polycyclic aromatic hydrocarbons (PAHs)

7.3.5.1 Poultry meat processing

The left pectoral muscles (50 g) were uniformly grilled at a temperature of 150^{o}C for 1 hour, (until there was good browning) inside a small electrically operated bachelor griller the temperature inside being determined by inserting a probe. The grilled meat samples were cooled, wrapped in aluminium foils, sealed and stored frozen (-20^{o}C) till time for further analysis.

7.3.5.2 Reagents and Chemicals

All chemicals and reagents used were of analytical grade. Dichloromethane, n-hexane, potassium hydroxide, sodium sulphate (anhydrous), silica gel, acetonitrile, alumina and methanol used in the extraction and clean up were purchased from British Drug House (BDH, England) store in Kampala. Water (glass triple distilled) was obtained from the Department of Geology, College of Natural Sciences in Makerere University. Seven priority PAH standards including benzo (a) pyrene, benzo (b) fluoranthene, benzo (k) fluoranthene, indeno (1,2,3-cd) pyrene, dibenz (a,h) anthracene, benzo (g,h,i) perylene and chrysene $d_{12;}$ Supelco Inc. (Bellefonte, PA), Sigma Aldrich Co and filters (chromafil 0.45 μm NYL,W/Gme-Macherey-Nagel; GmbH & Co. KG) were purchased from Germany.

7.3.5.3 Extraction

The extraction and purification methods, with minor modifications, were similar to those described by Takatsuki et al., (1985), Chen et al., (1996), and Husain et al., (1997). The sample (10.0 g), homogenised with an ultra turrax in a 50 mL Falcon tube was transferred into a 250 mL flat bottomed round flask and mixed with surrogate working solution (5 ng/g; 50 µL). Methanolic caustic potash (90% v/v, 2N KOH; 50 mL) was added and the mixture boiled under reflux for one and half hours. The solution was cooled, mixed with n-hexane (50 mL) and refluxed gently for 15 minutes. Water (50 mL) was added and the mixture cooled and kept in a freezer (4^oC) for 1 hour to allow the two phases (n-hexane and aqueous) to separate. The hexane layer (top) was isolated.

Blanks and spiked samples were similarly extracted. Two spikes were used where one sample was taken in triplicate. The first replicate was analysed without spiking; the second replicate was spiked at a level of 2 ng/ g for each PAH: where the standard working solution (1µg/mL, 2 µL) was added to the 10 g sample and extracted as above; the third replicate was spiked at a level of 10 ng/g for each PAH, when the standard working solution (1µg /mL, 100 µL) was added to the 10 g sample and extracted as described earlier.

7.3.5.4 Sample clean-up

The hexane layer (25 mL) was evaporated until a final volume of 1mL at 40°C under a stream of nitrogen. Acetonitrile (10 mL) was added and the mixture further evaporated until the volume was <= 5 mL. Petroleum spirit (2 mL) was then added and the mixture kept for 2 hours in a freezer (4^oC). The petroleum spirit (containing most of the fats in the sample) was then removed. The acetonitrile layer was concentrated at 40°C under a stream of nitrogen until a final volume <= 1 mL. The concentrate was then eluted through a column packed with alumina (5 g) and anhydrous sodium sulphate (2 g) below and above the alumina; previously conditioned with n-hexane/dichloromethane mixture (1:2, v/v, 15 mL). The eluate was

collected in a 40 mL dionex vial, the concentration tube was rinsed with n-hexane/dichloromethane (1:2,v/v, 5 mL) and the rinsing transferred to the column. The column was also rinsed with two portions of n-hexane/dichloromethane (1:2, v/v, 5 mL) and the collected eluates concentrated at 40^{o}C under a stream of nitrogen to a volume of <= 400 µL. Recovery working solution (25 µL) was added and the mixture further diluted to a volume of 500 µL using n-hexane. The tube was vortexed and the solvent allowed to settle. The final extract was then transferred to an autosampler vial with insert.

7.3.5.5 Gas Chromatographic analysis

The Gas Chromatograph was an Agilent 6890 N network GC system equipped with a 5975 Mass Selective Detector and an Agilent 7683B, 10 µL syringe auto sampler. A Zebron, ZB-5MSi (5% phenyl-95% dimethylpolysiloxane), Ultra Inert 30 m x 0.25 mm i.d. x 0.25 µm film thickness column was used to separate the compounds. This was operated in a constant flow mode with an initial flow rate of 4.9 mL/minute, initial pressure of 35.53psi and average velocity of 81cm/sec. The oven was initially set at 55°C for 1 minute, ramped to 320 °C at 25 °C/min and held for 3 minutes. Inlet was by pulsed, 1.0 µL splitless injection at an initial temperature of 300°C, pressure 35.53 psi until 0.2 minutes with purge flow of 30 mL/min and purge time 0.75 minutes. Carrier gas was helium (99.999%) at a constant flow rate of 45 cm/s. The Detector source was set at 300°C, quadrupole at 180°C, transfer line at 280°C; scan range 45 to 450 AMU and electronic impact at 70eV with solvent delay of 3.75 minutes.

Identification was mainly based on the retention time match of the compounds in the samples against those of the calibration standards. Positive confirmation required a retention time match of ±1% (Samuel et al., 2010). A difference of 6 seconds in the retention time match could therefore still be within the retention time window. Comparison of the mass spectra of unknown peaks with those in the NIST 17 Mass Spectral Database (library search) (NIST 2017/2014/EPA/NIH) was also performed.

For quantification of the PAH compounds the absolute calibration method was used. A minimum of four concentration levels of each PAH ranging from 0.1ppm to 15 ppm were injected onto the GC-MS and the calibration curve for each standard was obtained by plotting peak area against concentration. The regression equation and coefficient of determination (R^2) were calculated. Each PAH in the sample was then quantified using the formula:

$$As(ppb) = (Xs \times 1000)/(Ws \times R)$$

Where *As* is the PAH concentration in the sample in (µg kg^{-1}), Xs is the concentration relative to the peak area in the injection volume (1µL), Ws is the mass of the sample extracted (in grams) and R is the recovery of PAH. The figure 1000 was used to convert the units to µg kg^{-1} Xs on the other hand was computed by rearranging the regression expression:

$$y = a + bx, \text{ to give } x = (y-a)/b.$$

7.4 Quality control

To prove that the analytical protocol was under control and that no unrecognized changes during analysis influenced the analytical results the following were done.

7.4.1 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection and Limit of Quantitation are fundamental elements of method validation that define the limitations of an analytical method. In general, LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified under the stated conditions of the test (Alankar & Vipin, 2011). The IUPAC defines limit of detection as the smallest concentration or absolute amount of analyte such that the probability of type 1 and type 2 errors are equal. By convention and from statistics the limit of detection is the concentration corresponding to a signal three times the noise level of the background. LOQ on the other hand is generally taken as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the

stated conditions of the test. It is mathematically taken as equal to ten times the standard deviation of the results for a series of replicates used to determine a justifiable LOD. It may also be taken as ten times the signal to noise ratio. Both LOD and LOQ are matrix, method and analyte specific (Harvey, 2000).

In this study, both LOD and LOQ were computed based on the procedures of the International Conference on Harmonization (ICH) and EURACHEM guidelines namely, linear regression; (Alankar & Vipin, 2011; EURACHEM, 1998; ICH-Q2B., 1996; Marsin et al., 2000). The equation for the linear regression curve can be expressed in a model such as:

$$y = a + bx$$

Where **a** is the intercept of the graph, **b** the slope, **x** the analyte amount injected and **y** the instrument response.This model can be used to compute the sensitivity **b** and both the LOD and LOQ from the two expressions:

$$LOD = \frac{3.3St}{b} \text{ and } LOQ = \frac{10St}{b}$$

Where *St* is the standard deviation of the response and can be estimated from either the y-residuals S_{res} or y-intercepts, S_{yo} of the regression lines (Alankar & Vipin, 2011). In this particular case the standard deviations were computed using the y-intercepts.

7.4.2 Recovery Studies, Precision and Accuracy

Recovery studies were done to determine the extraction and clean up efficiencies of the methods used to extract and clean up the extracts from the samples. A set of three aliquots of homogenized breast muscles were spiked with the recovery solution (25µL) containing a mixture of the 6 PAH standards of concentrations (0 ng/mL, 2 ng/mL and 10 ng/ mL). Both the spiked (experimental) and the unspiked (control) aliquots were then taken through the same analytical procedures described earlier on. The percentage recoveries were calculated by comparing the concentrations in the spiked and control sample aliquots as follows:

$$\text{Recovery, \% R} = \frac{Cs - Cu}{Cn} \times 100$$

Where *Cs* is the concentration of the PAH in the spiked sample aliquot; *Cu* is the concentration of PAH in the unspiked (control) sample aliquot; *Cn* is the nominal (theoretical) concentration increase that results from spiking the sample. Precision, (repeatability) was estimated in terms of relative standard deviations from the recovery experiments at the stated fortification levels. The percent recoveries obtained were also used to estimate the accuracy (reproducibility) of the analytical method.

7.4.3 Calibration of the Detector

Prior to GC-MS analysis, the compound perfluorotributylamine (PFTBA) was used for calibration of the detector. PFTBA is preferred because of the mass range, the even spacing of the major fragments, and the volatility of the gas under the analyzer vacuum. The major m/z for the calibration compound are; 69, 131, 219, 264, 414, 464, 502, and 614. Key values are m/z of 69, 219 and 502, or the isotopes at 70, 220 and 503 respectively. The base mass is 69; fragments 131 and 219 have roughly the same heights, equal to 45-60 % of the 69 peak; the 414 peak is about 3-6 % of the 69 peak; and 502 will be 3% or less than 69 peak height.

The system was also tested for possibility of leak; where it was found that the ratio of 18 to 69 was only 2.39% (i.e. < 20%), while the ratio of 28 (N_2) to 69 was also found to be only 4.91% (i.e. < 10%). Electron multiplier voltage was reported at 1,188eV (i.e. < 2,000eV). All these were proof that the MS system was vented and the ion source was clean hence reliable results would be expected from the detector (McMaster & McMaster, 1998)

7.5 Statistical analysis

The randomly assembled data were analyzed by one-way ANOVA using the SPSS package (SPSS/PC1Statistics 18.0; SPSS Inc., Chicago, IL, USA, 2010). Scheffé's test was used for comparing mean values. However, data on PAHs were analysed to determine

whether any statistically significant differences existed between the data sets for the three groups. A Levene test for the homogeneity of variance and a one factor analysis of variance to test for equality of means (Levene, 1960), indicated statistically significant differences. The results showed that the PAH data sets could not be pooled and treated as a single data set for further statistical analysis. The data was then analysed using Kruskal Wallis H, the alternate non-parametric test to the oneway ANOVA.

Chapter 8

RESULTS AND DISCUSSIONS

8.1 Live weights and carcass traits of Cobb 500 broiler chickens

Final live weights and carcass traits of the Cobb 500 broiler chickens are shown in Table 8.1.

Table 8. 1 Mean values for final live weights and carcass traits of Cobb 500 broiler chickens.

	Group[1]				P-value
Trait	C	T1	T2	SEM	
Final live weight (g)	1328.23	1335.49	1300.52	24.89	0.842
Carcass weight (g)	857.42	918.09	924.70	19.69	0.317
Carcass yield (%)	64.49^{B}	68.79^{A}	71.03^{A}	0.76	0.000
Breast weight (g)	119.12	133.07	130.77	5.86	0.598
Breast yield (%)	13.91	14.52	14.10	0.58	0.913
Legs weight (g)	210.54	235.88	235.00	7.43	0.297
Legs yield (%)	24.49	25.65	25.55	0.64	0.732
Back weight (g)	264.52	271.31	253.74	6.92	0.595
Back yield (%)	30.99	29.54	27.62	0.68	0.125
Wings weight (g)	79.04	95.45	93.03	4.45	0.276
Wings yield (%)	9.19	10.52	10.13	0.49	0.544

[1]C = control; T1 = 0.25 mL (12.5 IU) of dl-α tocopheryl acetate; T2 = 0.5 mL (25 IU) of dl-α tocopheryl acetate. SEM: standard error means. A, B: $P < 0.01$.

The results indicate that intramuscular vitamin E injection did not have significant effect ($P > 0.05$) on the final live weights of the birds. However, the results show slightly higher ($P > 0.05$) mean carcass weights for both groups T1 (918.09 g) and T2 (924.70 g) compared to group C (857.42 g). Whereas these increases were not statistically significant, they translated into highly significant ($P < 0.01$) differences in the carcass yields; being higher in T2 (71.03 %) and T1 (68.79 %) groups compared with C group (64.49 %). On the other hand, the weights of breast, legs, back and wings, as well as their percentages were similar among the experimental groups ($P > 0.05$).

Apart from the statistically significant carcass yields observed in this study, most studies on the effect of vitamin E supplementation in diets on growth performance and carcass traits such as those performed by Leonel et al., (2007) and Adebiyi et al., (2011) gave similar results. Furthermore, these findings lend support to many results of similar studies carried out on other livestock species, such as pigs, (Niculita et al., 2007), bulls (Neto et al., 2012) and lambs (Atay et al., 2009), fed diets supplemented with vitamin E, as well as lambs intramuscularly injected with vitamin E (Maiorano et al., 2007). Similarly, Pompeu et al.,(2018), through a meta-analysis involving a database of up to 51 scientific papers published in peer reviewed journals, concluded that there seemed to be no relationship between dietary vitamin E supplementation and growth performance of broilers. However, these authors noted that there was ample indication that meat quality and immune response could be improved by dietary vitamin E supplementation. Furthermore, a study by Karadas et al., (2016), on the effects of different types of antioxidant (Selenium, vitamin E and carotenoids) on growth performance, skin pigmentation and liver concentrations of these antioxidants, concluded that, whereas selenium and vitamin E supplementation significantly improved total carotenoid concentrations in the plasma, which carotenoids enhanced skin and meat colour, none of the supplements tested influenced growth ($P > 0.05$), of broilers.

8.2 Physico-chemical properties of breast muscles of Cobb 500 broilers

The results for the physico-chemical analyses and total lipids of the breast muscles of Cobb 500 broiler chickens are presented in Table 8.2. Ultimate pH was not affected ($P > 0.05$) by vitamin E treatment, ranging from 5.62 to 5.70. On the contrary, treatment with vitamin E improved the water holding capacity of the breast muscles compared with the control, however the reduction of the water loss was more marked with higher dose of vitamin E (T2; $P < 0.01$). In this study, the pH_u and water holding capacity values were generally within the

acceptable range (Fletcher, 1999; Van Laack et al., 2000) for good quality meat; in addition, these values evidenced no pre-slaughter stress.

Table 8. 2 Mean values for pH, water holding capacity and total lipids of breast muscle of Cobb 500 broiler chickens

Trait	Group[1]			SEM	P-value
	C	T1	T2		
pH_{24}	5.70	5.63	5.62	0.03	0.533
Water holding capacity,%	12.44^{B}	11.19^{AB}	10.53^{A}	1.48	0.009
Total lipids,%	2.18	2.13	2.25	0.076	0.826

[1]C = control (0.5 mL physiological saline); T1 = 0.25 mL (12.5 IU) of dl-α tocopheryl acetate; T2 = 0.5 mL (25 IU) of dl-α tocopheryl acetate.
SEM: standard error means.
A, B: $P < 0.01$

pH and WHC are important indicators of the technological quality of meat that directly or indirectly affect consumer satisfaction with the final product. Poor WHC in raw poultry meat results in diminished visual appeal and inferior palatability traits for consumers as well as reduced ingredient retention, protein functionality, and saleability for processors (Bowker & Zhuang, 2013). Further, pH influences the microbiological shelf life, the extent of exudation as well as meat colour. A large extent of pH decline takes the muscle proteins to their isoelectric point (Huff-Lonergan & Lonergan, 2005). This loss in net electric charge reduces the repulsion between neighbouring protein molecules. The proteins pack more closely together forcing immobilized water as exudates into the free water compartments. A very low WHC results in Pale, soft and exudative (PSE)-like meat (Petracci et al., 2015). While, a very small extent of pH decline (high pH_u) leads to very high WHC and gives rise to dark, firm and dry (DFD) meat products (Huff-Lonergan & Lonergan, 2005). The addition of vitamin E to diets as well did not appear to affect pH values of meat from other livestock species as reported for: cattle (O'Grady et al., 2001) and pigs (Guo et al., 2011). On the other hand, Lauridsen et al., (1999), observed increased pH_u in pigs fed 200 mg of dl-α-tocopheryl

acetate/kg compared to pigs supplemented with 0 or 100 mg of dl-α-tocopheryl acetate/kg feed. Also Maiorano et al., (2007), reported that 1,200 IU of dl-α-tocopheryl acetate (150 IU/week) intramuscular injection of lambs raised the pH_u of longissimus muscle ($P < 0.05$). However, generally a difference in pH would not be expected because vitamin E has not been reported to directly affect glycolytic potential or postmortem glycolysis (Hasty et al., 2002).

However vitamin E treatment improved the water holding capacity of the breast muscles. This finding was similar to the report by Naik et al., (2015), who noted that Extract release volume (ERV) and water holding capacity (WHC) were significantly ($P < 0.01$) increased in organic selenium and vitamin-E supplemented groups of broilers as compared to control and was highest in birds fed 0.1ppm organic selenium with 300mg/kg vitamin-E. Literatures available (Grau & Ortiz, 1998; Pompeu et al., 2018) suggest that the improved water holding capacity and the good pH_u values could be due to the ability of vitamin E to protect muscle cell membranes. Studies have shown that vitamin E maintains the integrity of long chain polyunsaturated fatty acids in the membranes of cells and thus maintain their bioactivity. These bioactive lipids are important signaling molecules and changes in their amounts, or in their loss due to oxidation, or their oxidation products are the key cellular events that are responded to by cells (Farmer et al., 2006). The mechanism of α-tocopherol's protection of these lipids appears to be twofold: 1) the tocopherols' antioxidant action lowers the amounts of peroxylipids; the oxidized lipids increase the fluidity and packing errors of membranes, and thus would allow enzymes, such as PLA2, greater access to substrate phospholipids where they could cause destruction of cellular structures; 2) the α-tocopherol causes "repair" to membrane packing errors and thus inhibit PLA2 differently, but in this case without recourse to antioxidant activity (Grau & Ortiz, 1998). In both of these cases therefore the myofibrils may be kept intact or reorganized by repairing the packing error hence improving the water holding capacity of the meat.

Total lipids, ranging from 2.13% to 2.25%, were not significantly ($P > 0.05$) affected by vitamin E treatment at both concentration levels. The contents of intramuscular fat found in the present study were slightly higher than those reported in chickens slaughtered on 42 days by Maiorano et al., (2017) and Tavaniello et al., (2019), but similar to that reported by Gornowicz et al., (2009). Oxidative stability of the breast muscles was one other aspect evaluated in this study. Lipid oxidation is one of the primary causes of quality deterioration in meat and generates compounds potentially dangerous. The conversion of muscle to edible meat after slaughter can unbalance the equilibrium between pro-oxidative and anti-oxidative factors, resulting in initiation and propagation of lipid oxidation (Min & Ahn, 2005). Vitamin E treatment improved the oxidative stability of the pectoral muscle. The dose of 0.5 mL (25 IU) of dl-α tocopheryl acetate had a marked effect; in fact, the levels of TBARS were lower ($P < 0.05$) in group T2 (0.037 mg MD/kg) than in the control group (0.046 mg MD/kg) (Figure 8.1). The result obtained in this study appears to be in line with studies by Lohakare et al., (2005), who found significantly ($P = 0.0013$) lower values of TBARS in commercial broilers fed a combination of vitamin A (12,000 IU/ kg), vitamin E (100 IU/ kg) and methionine, and (Skřiva et al., (2010), who reported similar findings with diets supplemented with caprylic acid and vitamin E. Further, results from the work of Naik et al., (2015), found that significantly ($P < 0.01$) decreased level of TBARS and tyrosine value (TV) were observed in broilers fed organic selenium and vitamin-E compared to the control and was least in birds fed 0.1ppm organic selenium with 300 mg/kg vitamin-E.

Similar results on the effects of vitamin E treatments on lipid oxidation have been reported on other poultry species, such as ducks (Schiavone et al., 2010) and goose (Łukaszewicz et al., 2016); and also in other livestock species, such as pigs (Bahelka et al., 2011; Rossi et al., 2013) and lambs (Maiorano et al., 2015; Salvatori et al., 2004). Maiorano et al. (2015) attributed their results to the higher α-tocopherol content in muscles of treated

lambs, as was confirmed by the negative correlation (r = − 0.46) between TBARS and muscle α-tocopherol level. However, the TBARS values (ranging from 0.037 to 0.046 mg MDA/kg of meat) obtained in this study were similar to those previously reported for Ross broiler chickens by Maiorano et al., (2017), but lower than those found by (Castellini et al., 2006). Such differences could be due to different times and conditions of storage, and probably also to differences in the genotypes, feed formulation strategies and rearing conditions that may have direct influence on the intramuscular lipid contents of the breast muscles.

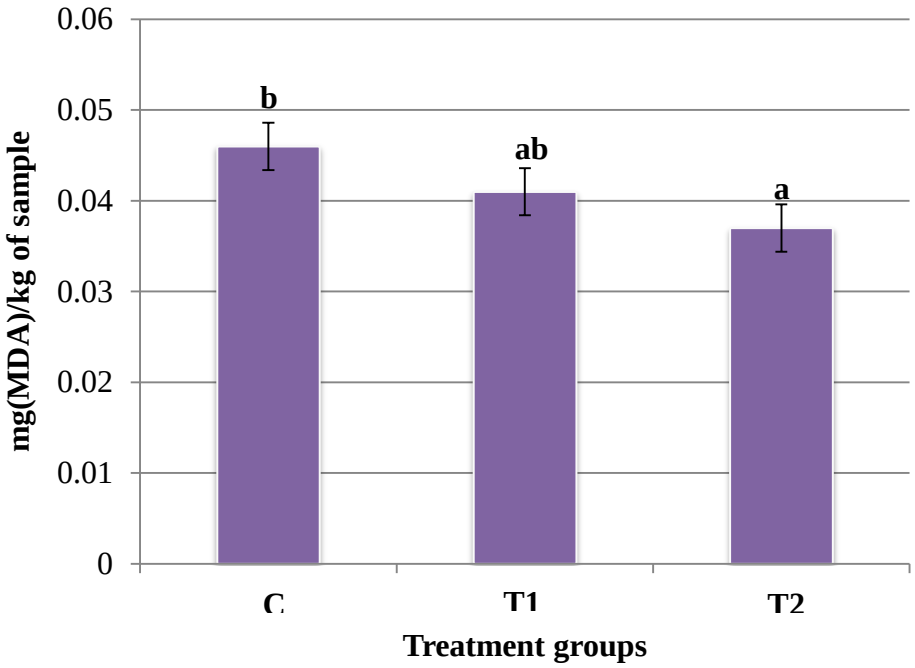


Figure 8. 1 Effects of vitamin E treatment on TBARS formation.
C = 0.5 mL physiological saline; T1 = 0.25 mL (12.5 IU), of dl-α tocopheryl acetate; T2 = 0.5 mL (25 IU) of dl-α tocopheryl acetate. TBARS expressed as mean mg (MDA)/kg of sample. a, b: $P < 0.05$.

8.3 Polycyclic aromatic hydrocarbon assay and food safety

8.3.1 Limit of detection and limit quantitation

As a measure of quality control, limits of detection and quantitation were also determined and are recorded in Tables 8.3. The detection limits for benzo(a)pyrene, benzo(b)fluorantene, benzo(g,h,i)perylene, benzo(k)fluoranthene, Dibenzo(a,h)anthracene

indeno(1,2,3-cd)pyrene were; 0.042, 0.063, 0.101, 0.051, 0.820, and 0.050 ng/mL (Table 8.3). These were good enough for determination of analytes at the μg/kg (ppb) levels.

Table 8. 3 Limits of detection (LOD) and Limits of quantitation (LOQ)

PAH Compound	LOD ng/mL	LOQ ng/mL
Benzo(a) pyrene	0.042	0.16
Benzo(b) fluoranthene	0.063	0.23
Benzo(g,h,i) perylene	0.101	0.36
Benzo(k) fluoranthene	0.051	0.18
Dibenzo(a, h) anthracene	0.820	2.80
Indeno (1,2,3-cd)pyrene	0.050	0.18

LOD were taken as 3times the Signal/Noise ratio; while LOQ = 10 x Signal/Noise ratio. Samples were considered positive when their analyte levels were ≥ LOD and were considered quantifiable when their analyte levels were ≥ LOQ.

8.3.2 Coefficient of determination, calibration range and recoveries.

Recovery and calibration studies were also performed for the six PAHs and the results are presented in Table 8.4.

Table 8. 4 Percent recoveries, CoD and calibration range of the 6 PAHs

PAH Compound[1]	Coefficient of Determination (R^2)	Calibration range μg/mL	Mean Percent recovery ±RSD (n=3)
BaP	0.9999	1.00-15.00	82.91±0.71
BbF	0.9981	1.00-10.00	84.57±3.05
BgP	0.9970	1.25-10.00	80.37±3.85
BkF	0.9975	1.00-10.00	82.23±3.51
DaA	0.9995	0.40-10.00	75.20±1.62
IcP	0.9970	0.40-10.00	77.08±3.86

[1]BaP = benzo (a) pyrene, BbF = benzo (b) fluoranthene, BgP = benzo(g,h,i) perylene, BkF = benzo (k) fluoranthene, DaA = dibenzo (a,h) anthracene, IcP = Indeno (1,2,3 cd) pyrene.
n = number of replicate analyses: RSD = Relative Standard Deviation; CoD = coefficient of detemination.

The coefficients of determination (CoD; R^2 values) of the standard curves between the peak area and concentrations of the 6 PAHs as shown in Table 8.4 were all above 0.99. Except for the recoveries of DaA (75.20 ±1.62%) and IcP (77.08 ± 3.86%) which were lower, the rest of

the recoveries were all above 80%. In actual sense, the recoveries of PAHs were in the range of (75.20 – 84.57%), with a mean recovery of 80.39 ± 3.72. These results were satisfactory for determinations at µg/kg levels (Jenke, 1996). Research has shown that sometimes the recoveries of some PAHs may be low because PAHs might undergo partial loss during the extraction and purification stage. According to Mottier et al., (2000), a principal loss normally occurs due to partitioning of BaP and other PAHs, into the alkaline methanolic saponification solvent. Takatsuki et al., (1985), added that such partitioning leads to formation of emulsions that are difficult to breakdown hence lowering the amount of free BaP. They further observed that light might also degrade PAHs during extraction. In a study by Russo et al., (2016), to determine the effect of pH on PAH recoveries from beer samples, two real beer samples were spiked with 5 ppm of each PAH; recoveries obtained at pH9 (23.5 - 59.0%) were much lower than those obtained at pH4 (85.3 - 102.7%). This occurrence, Russo et al., (2016) attributed to formation of a gel on adding the strong alkali (sodium hydroxide), that adsorbed the analytes and lowered the recoveries. Since the extraction of PAHs from meat matrices usually involves saponification with methanolic caustic potash, the resultant high pH may therefore lead to gel formation that lowers the recoveries of the analytes. It has also been established that the presence of impurities, such as aliphatic hydrocarbons, fatty acids, phenols and polycyclic organic compounds, may influence the extraction recoveries of PAHs (Chen & Lin, 1997).

EPA Method 8000c (2003), proposed that for methods without recommended acceptance criteria the recoveries of compounds spiked into samples should fall within 70-130% and this range should be used as a guide in evaluating in-house performance. While Directive 2005/10/EC adopted by EU requires that only validated methods that yield at least 50-100% recoveries should be used for analysis of BaP contamination in food groups (Stadler & Lineback, 2009). The analytical protocol employed in this study therefore meets both these

two requirements. Thus the recoveries and coefficients of determination obtained in this study were suitable for determination of PAHs at the μg/kg level.

8.3.3 Polycyclic aromatic hydrocarbon concentrations

The concentrations of the PAH compounds that were present in the samples at or above their limits of quantitation are shown in Table 8.5. Six PAH standards were acquired and used for the identification and quantification of the PAH compounds formed during grilling of the breast muscles. The results in Table 8.5 indicate that, of the six PAHs that were able to be identified and quantified, IcP had the highest frequency of occurence (08) and DaA the least frequency (01), of all the samples analysed. In quantitative terms, the results show that where it was detected and quantitated, BkF has been detected at moreless higher amouts (4, 8, 12 and 13 μg/kg) compared to most of the other PAHs detected. The results also indicate that higher frequencies of occurrence of the mutagens were reported in the control group (17) compared to the two treatment groups T1 (08) and T2 (05) respectively. BaP, the marker for the carcinogenic potency of PAHs was detected in only 1 sample from the tretment groups (T1) as opposed to (05) in the control group. Further, when detected, the concentration of BaP was always within the EU recommended limit (5μg/kg) except in one sample where it was slightly higher (6.78 μg/kg).

Theoretically an ideal separation technique should allow for complete resolution of all the 16 PAHs with low detection limits, high reproducibility, high sensitivity and short retention time. Gas Chromatography is often used in the separation of complex PAH mixtures in various food matrices because of its superior column efficiency and ability to provide independent confirmation of the compounds (Jira et al., 2008). Three major problems associated with the extraction and analysis (identification and quantification) of PAHs from food matrices however are: 1) The infinitesimal amounts in which PAHs normally occur in food matrices; 2) The presence of many PAH like impurities that can be co-extracted with

PAHs from the food and 3) The existence of PAHs in isomeric forms. Choice of the methods of extraction, clean up and analysis should always be with the above in mind so that quantitative results can be realized (Chen et al., 1997).

The mean concentrations of the six PAHs identified and quantified from the samples (Table 8.5) are also illustrated in Figure 8.2. As earlier explained under statistical analysis, the data set for this determination failed the Levene test for the homogeneity of variance and the one Factor analysis of variance to test for equality of means (Levene, 1960). The data was therefore analysed using Kruskal Wallis H test, the alternate non parametric test to the one way ANOVA. The test showed that there was statistically no significant difference in the means between the three groups: χ^2 (2) = 0.467, P = 0.792 with a mean rank pair of 16.90 for the control, 14.70 for T1 and 14.90 for T2.

Table 8. 5 PAH concentrations and frequency of detection

Group[1]		Mean concentrations of PAHs[2] (μg/kg)						
		BaP	BbF	BgP	BkF	DaA	IcP	ΣPAHs
C (n = 10)	Min	1.14	1.57	4.24	nd	1.25	1.06	9.26
	Max	6.78	4.14	7.68	8.91	6.13	6.71	40.35
	Mean	1.55	0.57	1.19	0.89	0.94	1.18	6.32
	Freq	*05*	*02*	*02*	*01*	nd	*04*	*14*
T1 (n = 10)	Min	nd	nd	nd	2.81	nd	2.48	5.29
	Max	2.99	3.66	8.03	12.17	nd	13.17	40.02
	Mean	0.30	0.37	0.80	1.97	nd	1.57	5.01
	Freq	*01*	*01*	*01*	*03*	*nd*	*02*	*08*
T2 (n = 10)	Min	nd	2.19	nd	nd	nd	2.56	4.75
	Max	nd	14.78	nd	13.41	nd	9.87	38.06
	Mean	nd	2.54	nd	1.34	nd	1.24	5.12
	Freq	nd	*03*	nd	*01*	nd	*02*	*06*

[1]C = 0.5 mL physiological saline; T1 = 0.25 mL (12.5 IU) dl α- tocopheryl acetate; T2 = 0.5 mL (25 IU) dl α- tocopheryl acetate. [2]BaP = benzo (a) pyrene, BbF = benzo (b) fluoranthene, BgP = benzo(g,h,i) perylene, BkF = benzo (k) fluoranthene, DaA = dibenzo (a,h) anthracene,

IcP = Indeno (1,2,3 cd) pyrene. Results are means of duplicate analyses. Freq = frequency of detection (out of 30). nd = not detected.

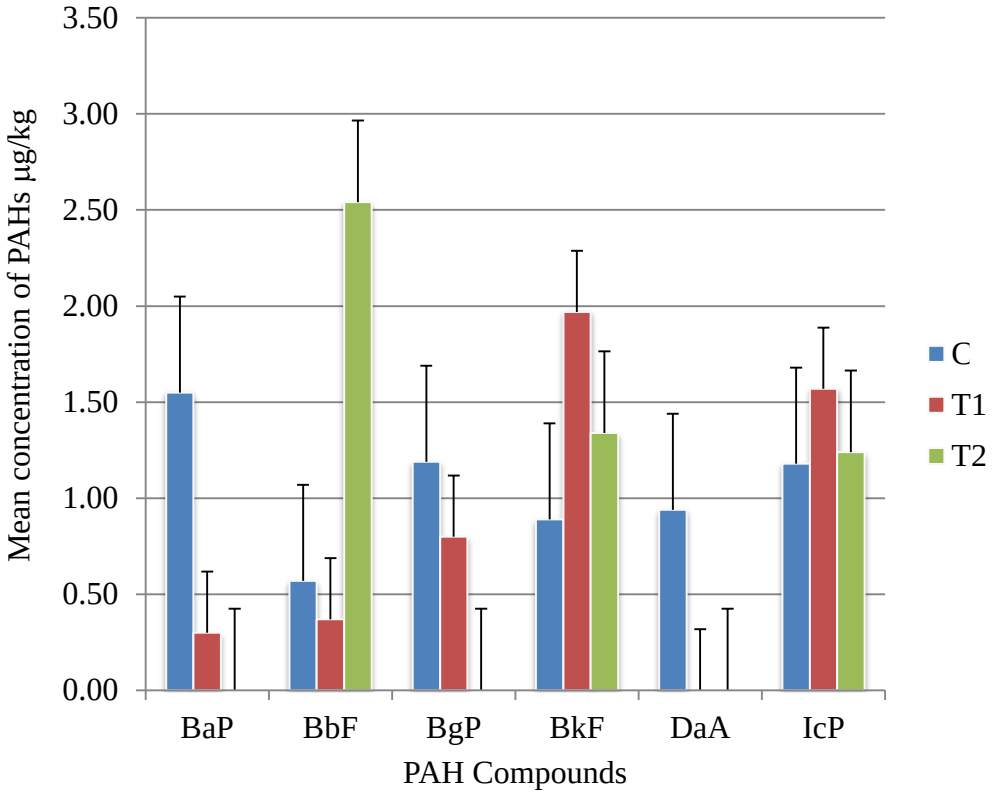


Figure 8. 2 Concentrations (µg/kg) of PAH compounds formed

C = 0.5 mL physiological saline; T1 = 0.25 mL (12.5 IU) dl α-tocopheryl acetate; T2 = 0.5 mL (25 IU) dl α- tocopheryl acetate. Bap = benzo (a) pyrene, BbF = benzo (b) fluoranthene, BgP = benzo (g,h,i) perylene, benzo (k) fluoranthene, DaA = dibenzo (a, h) anthracene, IcP = indeno (1,2,3-cd) pyrene

The mean concentrations (µg/kg) of the compounds formed in the samples were in the range BaP (0.30 – 1.55), BbF (0.37 – 2.54), BgP (0.80 – 1.19), BkF (0.89 – 1.97), DaA (0.94) and IcP (1.18 – 1.57). These results were similar to those obtained by Janoszka etal., (2004), who worked on thermally treated high protein food (chicken), and obtained mean concentrations of: BkF (0.21), BaP (0.15) and BgP (0.63) µg/kg, and also to the result of (El-Saeid, 2010) that ranged from 0.12 to 2.55 µg/kg for the six PAHs and those obtained by Chung et al., (2011), who obtained mean concentrations (µg/kg) as; BbF (2.15), BkF (0.56), BaP (1.90), DaA (0.14), BgP (1.44) and IcP (0.85) from charcoal grilled barbecued chickens.

The results are however slightly different from those obtained by EL-Bardy, (2010), who found that, of the six PAH compounds (BbF, BkF, BgP) were not detected in all the grilled chicken samples while the other three were present at slightly higher mean concentrations; BaP (3.8), IcP (5.5) and DaA (12.12) μg/kg.

In this study the content of benzo (a) pyrene (the marker) in 30 chicken breasts were determined by the GC-MS method. BaP contents in breast muscles were in the range of < 0.16 and 6.70 μg kg^{-1} with a mean concentration of 0.3 μg kg^{-1} for group T1 (0.25 mL, 12.5 IU) and 1.55 μg/kg for the control group with no detection for group T2 (0.5 mL; 25 IU) The mean concentration of BaP was therefore within the tolerable limit set up by EU regulation (5 μg/kg). The results were similar to that reported by Hamzawy et al., (2016), who found that Benzo(a)Pyrene concentration in charcoal grilled chicken ranged from 0.49 to 7.20 μg/kg with mean concentration of 2.01 μg/kg. In 80% of the analyzed samples BaP concentrations remained below the limit of detection (< 0.042 μg kg^{-1}) and the maximum acceptable concentration of 5μg kg^{-1} for BaP in the grilled chicken products (European Commission, 2005) was exceeded in only 1 sample (6.7μg/kg). Simko (2002), reported concentrations of BaP in the range of 0.03-1.00 μg kg^{-1}, while in cooked meat products a concentration of 0.11-3.93 μg kg^{-1} of BaP have been detected (Janoszka et al., 2004).

The results of this research was similar to the work of Ruan et al. (2014), who worked on grilled lean beef evaluating the effect of vitamin E dietary supplementation on formation of heterocyclic aromatic amines and found that the total content of heterocyclic amines (HAs) in grilled lean beef ranged from 9.57 ng/g to 11.59 ng/g. There was, however, a trend ($P = 0.097$) found for reduced mutagenicity with increasing tissue levels of α-tocopherol. The increasing dietary vitamin E significantly increased the α-tocopherol level in lean beef ($P < 0.001$), but it had no significant ($P > 0.05$) inhibitory effects on the content of individual and total HAs. However, whereas the report of Ruan et al., (2014) and the result of this study

indicate no significant effect of vitamin E treatment on formation of carcinogenic compounds during grilling of meat, there appears to be some apparent trend towards inhibitory effects of vitamin E treatment on formation of PAHs. This is evidenced by the fact that, in this study all the six compounds were detected in the control group, while only five and three of the compounds were detected in group T1 and group T2 respectively (Figure 8.2), with the highest concentration of BaP reported in the control group. This result therefore appears to suggest that probably if the concentration of vitamin E was above what was used in this study or if a higher sample size had been used, there probably could have been a significant difference between the means of the different groups.

Figure 8.2 however shows that the concentrations of BbF, BkF and IcP reported for the vitamin E treated groups were much higher than for the control group. This could simply be due to the fact that there are always some separation problems occurring between the pairs of PAH compounds: BkF/BbF, IcP/DaA and to a lesser extent between BaA and Chr. This has always been attributed to the similarity in molecular weights, boiling points and chemical behaviour of the said compounds. Moreover, reports indicate that BbF normally co-elutes with another of its isomer, BjF which was not studied in this case. However, the fact that these occurrence was more prominent in the vitamin E treated groups compared to the control group is subject to further investigation.

However because BaP is not the only possible carcinogenic compound among the 16 priority PAHs, the concept of toxic equivalency was applied to judge the safety levels of the samples analysed vis-à-vis the PAH concentrations determined.

8.3.4 Toxic equivalency as a measure of carcinogenic potency

The fact that BaP was detected at very low concentrations in the samples analysed was no proof that the samples were safe for human consumption. The concept of BaP equivalent concentration commonly referred to as the toxic equivalency (TEQ) was employed. This

calculation needed the use of toxic equivalent factors (TEFs). The concept is based on the assumption that there is reasonably well characterized reference compound, qualitative similar toxic effects for all members of the class, and the toxic effects of different compounds are additive. A list of TEFs completed by (Nisbet and LaGoy, 1992) was used to estimate the carcinogenic potency of total PAHs (i.e. total BaP equivalent concentration) using the formula:

$$TEQ = \Sigma(PAHi \times TEFi)$$

Where TEQ is the toxic equivalents of the reference compounds.

$PAHi$ is the concentration of PAH congener i .

TEFi is the toxic equivalent factor for PAH congener i.

The computed TEQ was then compared with the maximum limit set by Regulation (EC) 1881/2006 and Regulation (EU) 835/2011 (an amendment to Regulation (EC) No 1881/2006). The TEF values used in the calculation are given in Table 8.6.

Table 8. 6 Toxic equivalent factor for individual PAH studied (Nisbet and LaGoy, 1992)

PAH	TEF	PAH	TEF
Benzo[a]pyrene	1.0	Benzo[b]fluoranthene	0.1
Benzo[k]fluoranthene	0.1	Benzo[g,h,i]perylene	0.01
Dibenzo[a,h]pyrene	1.0	Indeno[1,2,3-cd]pyrene	0.1

The results for such computation are shown in Table 8.7 from which it can be seen that the total TEQ for all samples considered for the three groups was 3.98 µg/kg with a mean of 1.33 µg/kg. Both the total and the mean were within the acceptable limit set for BaP level in EC Regulation (EC) No 1881/2006 and (EU) 835/2011 for muscle meat (Appendix 2). The sum of TEQ for each of the groups considered were 2.77 µg/kg, 0.702 µg/kg and 0.51µg/kg for the control, for T1 and T2 respectively. All these values were within the limit for BaP as required by EC Regulation (EC) No 1881/2006 and (EU) 835/2011, still in force (Appendix

2). However, aware of the fact that some of the compounds could have simply been degraded during saponification (Mottier et al., 2000) and could have actually added to these TEQ values, there is need to monitor the levels of PAHs especially the high molecular weight (HMW) components in food samples.

Table 8. 7 PAHs mean content (µg/kg) and BaPeq in samples from groups C, T1and T2

	Toxic Equivalency				
PAH	C	T1	T2	∑TEQ	Mean TEQ
BaP	1.55	0.30	0.00	1.85	0.62
BbF	0.057	0.037	0.254	0.348	0.12
BgP	0.012	0.008	0.00	0.02	0.01
BkF	0.09	0.20	0.13	0.42	0.14
DaA	0.94	0.00	0.00	0.94	0.31
IcP	0.118	0.157	0.124	0.40	0.13
∑TEQ	2.77	0.702	0.51	3.98	1.33

C = 0.5 mL physiological saline; T1 = 0.25 mL (12.5 IU) dl α- tocopheryl acetate; T2 = 0.5 mL (25 IU) dl α- tocopheryl acetate.
n.d = not detected, BaPeq = BaP equivalent concentration TEQs were calculated on the basis of the content and toxic equivalent factors for the 6 PAHs detected.

Chapter 9

CONCLUSIONS

This study aimed at evaluating the effect of intramuscular vitamin E injection on growth performance, meat quality and safety of meat of Cobb 500 broiler chickens raised under tropical climatic conditions. On the whole the results obtained indicate that vitamin E treatment did not have any significant ($P > 0.05$) effect on live and carcass weights, and also on the leg, back + neck, wing weights and their corresponding yields. However, treatment with vitamin E according to the results obtained, greatly ($P < 0.01$) improved the carcass yields. The study has also shown no significant ($P > 0.05$), effect of the treatment on pH_{24}, and total lipids but significant improvement on the water holding capacity ($P < 0.01$) and oxidative stability ($P < 0.05$) of the breast muscles. Further this research has shown that whereas there appears to be no significant effect; ($\chi^2(2) = 0.467$, $P = 0.792$ with a mean rank pair of 16.90 for the (control group), 14.70 for (group T1) and 14.90 for (group T2) of the treatment with vitamin E on formation of PAH compounds during grilling of broiler breast muscles, an apparent trend towards inhibition of the formation of these carcinogenic compounds appeared to have emerged. This was evidenced by the fact that more of the compounds were detected in the control as opposed to the treatment sub groups. However, the fact that isomeric and similar molecular weight compounds were detected at much higher concentrations in the treatment groups calls for further investigation. The results from the Toxic Equivalent (TEQ) computation also revealed that the concentrations of the compounds detected were within the tolerable limit set by EU Regulation (EU) 835/2011, still in force. However, because only six of the possible sixteen carcinogenic PAHs listed by USEPA were considered in this study, there is need for constant monitoring for the levels of these carcinogenic compounds in food matrices. Further, it should be noted that the attempt to investigate the inhibitory effect of intramuscular vitamin E injection on the formation of

carcinogenic compounds in broilers has been probably the first in Uganda if not in the greater part of the world and should therefore be continued.

Further as explained in the discussion, there appears to be no fixed level of supplementation of vitamin E into the animals that is believed to be optimal as yet. Therefore, one of the main objectives of studies concerning the potential effect of vitamins in livestock animals should be establishing the optimal dietary dose or the more standardized intramuscular administration of those nutrients that may positively influence the health, level of production and quality of animals and their products. Insignificant effects of examined vitamin E on almost all the slaughter traits so far reported appeared to be connected with the inadequate amount of *dl*-α-tocopheryl acetate supplementation in the birds. More so, due to the high cost involved, many studies appear to use quite small sample sizes, though the reporting may be quite different. This greatly jeopardizes scientific evidence and should be discouraged and more appropriate sample sizes be used or else authentic (scientific) reporting be encouraged as we struggle to solve the problems in a scientific manner.

List of Publications

Tavaniello S., Mucci R., **Acaye O.**, Bednarczyk M., Maiorano G. (**2019**). Effect of *in ovo* administration of different synbiotics on carcass and meat quality traits in broiler chickens. *Poultry Science*, 98; 464-472, https://dx.doi.org/10.3382/ps/pey330

Petrecca V., **Acaye O.**, Tavaniello S., Mucci R., Prioriello D., Ondřej B., Maiorano G. Quality and safety of meat from wild boar hunted in Molise region. Submitted to *Animal.*

Maiorano G., Tavaniello S., Slawinska A., Bertocchi M., Zampiga M., Palazzo M., **Acaye O.** In ovo delivery of GOS in slow-growing broiler chickens exposed to heat stress: implications for meat quality traits. Accepted for the 23rd National Congress of the Animal Science and Production Association "New challenges in Animal Science", 11-14 June **2019**, Sorrento (Italy).

Tavaniello S., Mucci R., **Acaye O.**, Bednarczyk M., Maiorano G. (**2017**). Probiotic and prebiotic supplementation in broiler chickens: growth performance, carcass traits and meat quality. *Italian Journal of Animal Science* 16(**1**): 49 **ISSN** 1594-4077 http://www.tandfonline.com/tjas

Acaye O., Tavaniello S., Wilkanowska A., Mucci R., Angwech H., Maiorano G. (**2016**). The effects of rearing system and intramuscular vitamin E injection on growth performance and meat quality of broiler chickens. *Slovak Journal of Animal Science*, 49(**4**); 164.**ISSN** 1337 – 9984

REFERENCES

Aaslyng, M. D. (2002). Quality indicators for raw meat. In J. P. Kerry, J. F. Kerry, & D. Ledward (Eds.), *Meat Processing* (pp. 157–174). Cambridge.

Abedin, Z., Louis-Juste, M., Stangl, M., & Field, J. (2013). The role of base excision repair genes OGG1, APN1 and APN2 in benzo[a]pyrene-7,8-dione induced p53 mutagenesis. *Mutation Research*, *750*, 121–128.

Abid, Z., Cross, A. J., & Sinha, R. (2014). Meat, dairy, and cancer. *American Journal of Clinical Nutrition*, *100*(Suppl 1), 386S–893S.

Adebiyi, O. A., Adu, O. A., & Olumide, M. D. (2011). Performance characteristics and carcass quality of broiler chicks under high stocking density fed vitamin E supplemented diet. *Agriculture and Biology Journal of North America*, *2*(8), 1160–1165.

Agerstad, M. J., & Skog, K. (2005). Review genotoxicity of heat-processed foods. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *574*, 156–172.

Alankar, S., & Vipin, B. G. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, *2*(1), 21–25.

Albert, R. E. (1983). Comparative carcinogenic potencies of particulates from diesel engine exhausts, coke oven emissions, roofing tar aerosols and cigarette smoke. *Environmental Health Perspectives*, *47*, 339.

Alexandratos, N., & Bruinsma, J. (2012). *World agriculture towards 2030/2050: the 2012 revision Rome, FAO: ESA Working paper* (No. 12-03). Rome.

Aliani, M., & Farmer, L. J. (2005). Precursors of chicken flavour II:Identification of key flavour precursors using sensory methods. *Journal of Agricultural Food Chemistry*, *53*, 6455–6462.

Alm El-Dein, A. K., Soliman, M. M., & Abd El-Aziz, S. (2013). Effect of dietary vitamin e supplementation on some productive, reproductive and immunological traits of inshas chickens strain. *Egyptian Poultry Science Journal*, *33*(IV), 939–955.

Alonge, D. O. (1988). Carcinogenic polycyclic aromatic hydrocarbons (PAH) determined in Nigerian kundi (smoke-dried meat). *Journal of the Science of Food and Agriculture*, *43*(2), 167–172.

Alum, E. A., Urom, S. M. O. C., & Ben, C. M. A. (2016). Microbiological Contamination Of Food: The Mechanisms, Impacts And Prevention. *International Journal of Scientific & Technology Research*, *5*(3), 65–78.

Alvarado, Z., & Owens, C. M. (2005). *Poultry: chemistry and biochemistry. In: Handbook of Food Science, Technology, and Engineering*. (Y. H. Hui, Ed.). Taylor & Francis Group.

Amiri Andi, M., Sharazed, M., Pourbakhsh, S. A., Afshar, M., Rokni, H., Shiri, N. E., & Salahi, Z. (2006). Effects of vitamin E in broiler breeder diet on hatchability, egg quality and breeder and old day immunity. *Pakistan Journal of Biological Sciences*, *9*(5), 789–794.

AMS. (1995). *Poultry Grade Yield Report, Poultry Grading Branch*. Washington, DC.

Anne, G., James, P. M., & Arthur, V. (1999). The secondary structure of type I collagen N-telopeptide as demonstrated by Fourier transform IR spectroscopy and molecular modeling. *Proceedings of the Indian Academy of Science*, *111*(1), 121–131.

Antwi-Boateng, C., Owusu-Prempeh, V., & Yeboah-Asuamah, S. (2013). Assessment of factors influencing the consumption of pork and poultry meat in Ghana: a comparative study. *International Journal of Innovative Research in Management*, *2*(4), 1–12.

Applegate, J. T. (2014). Protein and amino acid requirements for poultry.

Aronal, A. P., Huda, N., & Ahmad, R. (2012). Amino acid and fatty acid profiles of peking and muscovy duck meat. *International Journal of Poultry Science*, *11*(3), 229–236.

Atal, A., Levendis, Y. A., Carlson, J., Dunayevskiy, Y., & Vouros, P. (1997). On the survivability and pyrosynthesis of PAH during combustion of pulverized coal and tire crumb. *Combustion and Flame, 110*, 462–478.

Atay, O., Gökdal, Ö., Eren, V., Çetiner, Ş., & Yilkimaz, H. (2009). Effects of dietary vitamin E supplementation on fattening performance, carcass characteristics and meat quality traits of Karya male lambs. *Archiv Tierzucht*, *52*(6), 618–626.

ATSDR. (1995a). *Toxicological profile for polycyclic aromatic hydrocarbons (PAHs) (Final Report)*. Atlanta.

ATSDR. (1995b). *Toxicological profile for polycyclic aromatic hydrocarbons (PAHs) (Public health statement)*. Atlanta, GA.

ATSDR. (1996). *Polycyclic Aromatic Hydrocarbons (PAHs)*.

ATSDR. (2009). *Toxicological profile for polycyclic aromatic hydrocarbons (PAHs)*. Retrieved from http://www.atsdr.cd.gov/csem.

Aug, K. (1865). Sur la constitution des substances aromatiques. *Bulletin de La Societe Chimique de Paris.*, *3*(2), 98–110.

Augustyniak, A., Bartosz, G., Čipak, A., Duburs, G., Horáková, L., Łuczaj, W., & Žarkovic, N. (2010). Natural and synthetic antioxidants: An updated overview. *Free Radical Research*, *44*(10), 1216–1262.

Australia. (2009). *Polycyclic aromatic hydrocarbons: Health effects*. Retrieved from www.health.sa.gov.au/pehs/environ- health-index.htm

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity, 2014*, 360438.

Babij, A. S., & Kee, G. S. (1994). Changes in sarcoplasmic and myofibrillar proteins of spent hen and broiler meat during the processing of surimi-like material (Ayami). *Pertanika Journal of Tropical Agricultural Science*, *17*(2), 117–123.

Babji, A. S., Froning, G. W., & Ngoka, D. A. (1982). The effect of preslaughter environmental temperature in the presence of electrolyte treatment on turkey meat quality. *Poultry Science*, *61*(12), 2385–2389.

Bachinger, H. P., Mizuno, K., Vranka, J., & Boudko, S. P. (2010). *Comprehensive Natural Products II: Chemistry and Biology*. (L. Mander & H. W. Liu, Eds.). Elsevier Ltd.

Bahelka, I., Nürnberg, K., Küchenmeister, U., & Lahučký, R. (2011). Chemical composition, meat quality and oxidative status of pork after supplementation of diet with vitamin e and/or vitamin e + herb extracts. *Biotechnology in Animal Husbandry*, *27*(3), 853–860.

Bates, R. G. (1973). *Determination of pH: theory and practice*. Wiley.

Bedford, D., Claro, J., Giusti, A. M., Karumathy, G., Lucarelli, L., Mancini, D., & Yang, D. (2017). *Food Outlook; biannual report on global food markets*. Retrieved from www.fao.org/publications

Belova, A. V, Smutka, L., & Rosochatecká, E. (2012). World Chicken meat Market - Its development and current status. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *LX*(4), 15–30.

Bendall, J. R. (1980). *The electrical stimulation of carcasses of meat animals*. (R. Lawrie, Ed.). London: In: Developments in meat science, volume 1. Ed. R. Lawrie. Applied Science Publishers, London. 37-59.

Benford, D., DiNovi, M., & Setzer, R. W. (2009). Application of the margin-of-exposure (MoE) approach to substances in food that are genotoxic and carcinogenic e.g.: Benzo[a]pyrene and polycyclic aromatic hydrocarbons. *Food and Chemical Toxicology*, *48*(Supplement 1), S42–S48.

Benzie, P. (1996). Lipid peroxidation: A review of the causes, consequences, measurements and dietary. *International Journal of Food Science and Nutrition*, *47*, 233–261.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002a). *Fatty Acids Are Key Constituents of Lipids*. (M. J. Berg, J. L. Tymoczko, & L. Stryer, Eds.) (5th ed.). New York: W. H. and

Company. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK22497/ (cited 21st August 2018)

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002b). *Lipids and Cell Membranes. In: Biochemistry*. (M. J. Berg & L. J. Tymoczko, Eds.) (5th ed.). New York: Freeman W. H. and Company. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK22497/

Berri, C., Besnard, J., & Relandeau, C. (2008). Increasing dietary lysine increases final pH and decreases drip loss of broiler breast meat. *Poultry Science*, *87*, 480–484.

Best, B. (2001). Vitamin E (Tocopherols and Tocotrienols).

Bezkorovainy, A., & Rafelson, M. E. (1995). *Proteins with biological activity: Fibrous Proteins. In: Concise biochemistry*. (A. Bezkorovainy & M. E. Rafelson, Eds.). Marcel Dekker, Inc.

Bhattacharjee, A., & Bansal, M. (2005). Critical Review Collagen Structure : The Madras Triple Helix and the Current Scenario. *International Union of Biochemistry and Molecular Biology Life*, *57*(March), 161–172.

Bigolin, J., Weber, C. I., & da Trindade Alfaro, A. (2013). Lipid oxidation in mechanically deboned chicken meat: effect of the addition of different agents. *Food and Nutrition Sciences*, *4*, 219–223.

Binkova, B., Chvatalova, I., Lnenickova, Z., Milcova, A., Tulupova, E., & Farmer, P. B. (2007). PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair gene polymorphisms. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *620*(1–2), 49–61.

Bjrneboe, A. (1990). Absorption , Transport and Distribution of Vitamin E. *Journal of Nutrition*, *120*(May), 233–242.

Blaszczyk, I., Grucka-Mamczar, E., Kasperczyk, S., & Birkner, E. (2010). Influence of methionine upon the activity of antioxidative enzymes in the kidney of rats exposed to sodium fluoride. *Biological Trace Element Research*, *133*, 60–70.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Bopp, M., Lovelady, C., Hunter, C., & Kinsella, T. (2005). Maternal diet and exercise: effects on long-chain polyunsaturated fatty acid concentrations in breast milk. *Journal of American Dietary Association*, *105*, 1098–1103.

Bouts, T., & Gasthuys, F. (2003). The importance of vitamin E in zoo mammals. *Laams Diergeneeskundig Tijdschrift*, *72*, 125–129.

Bouyeh, M. (2013). Effects of excess dietary lysine and methionine on performance and

economical efficiency of broiler chicks. *Annals of Biological Research*, *4*(5), 241–246.

Bowker, B., & Zhuang, H. (2013). *Water holding capacity in poultry breast meat*. Athens.

Bragagnolo, N., & Rodriguez-Amaya, D. B. (2002). Simultaneous determination of total lipid, cholesterol and fatty acids in meat and backfat of suckling and adult pigs. *Food Chemistry*, *79*, 255–260.

Bramley, P. M., Elmadfa, I., Kafatos, A., Kelly, F. J., Manios, Y., Roxborough, H. E., & Wagner, K. H. (2000). Vitamin E. *Journal of Science Food and Agriculture*, *80*, 913–938.

Brunton, N. P., Cronin, D. A., & Monahan, F. J. (2002). Volatile components associated with freshly cooked and oxidized offflavours in turkey breast meat. *Flavour Fragrance Journal*, *17*, 327–334.

Burdge, G. C. (2006). Metabolism of α-linolenic acid in humans. *Prostaglandins Leukotrienes Essential Fatty Acids*, *75*, 161–168.

Burdge, G. C., & Calder, P. C. (2005). Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprodroduction Nutrition Development*, *45*, 581–597.

Bushby, R. J., & Lozman, O. R. (2002). Curr. Opinion on solid sate materials. *Science*, *6*(6), 569.

Byarugaba, D. K. (2007). *The structure and importance of the commercial and village based poultry industry in Uganda*. Kampala.

Byarugaba, D. K., E., O. J., & Katunguka-Rwakishaya, E. (2002). Production, Management and Marketing Dynamics of the Rural Scavenging Poultry in Uganda. In *Second FAO/INFPD Electronic Conference On Family Poultry 2002 on Bangladesh Model*. Retrieved from http://www.fao.or

Calaf, G., & Russo, J. (1993). Transformation of human breast epithelial cells by chemical carcinogens. *Carcinogenesis*, *14*, 483–492.

Calder, P. (2006). n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. *American Journal of Clinical Nutrition*, *83(suppl)*, 1505S–1519S.

Calvert, C. C., Desai, I. D., & Scott, M. L. (1964). Effect of linoleic acid on nutritional muscular dystrophy in the chick. *Journal of Nutrition*, *83*, 307–313.

Canan, H., Balci, F., & Udum, C. D. (2007). The Effects of Ozone and Chlorine Applications on Microbiological Quality of Chickens During Processing. *Journal of Biodiversity and Environmental Sciences*, *1*, 131–138.

Carrie, R. D., Amanda, J. C., Barry, I. G., Albert, R. H., Yikyung, P., & Rashmi, S. (2012).

Prospective investigation of poultry and fish intake in relation to cancer risk. *Cancer Preventive Research*, *4*(11), 1903–1911.

Castellini, C., Dal Bosco, A., Bernardinia, M., & Cyrilb, H. W. (1998). Effect of Dietary Vitamin E on the Oxidative Stability of Raw and Cooked Rabbit Meat. *Meat Science*, *50*(2), 153-161.

Castellini, C., Dal Bosco, A., Mugnai, C., & Pedrazzoli, M. (2006). Comparison of two chicken genotypes organically reared: oxidative stability and other qualitative traits of the meat. *Italian Journal of Animal Science*, *5*, 355–363.

Cavalieri, E. L., & Rogan, E. G. (1992). The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic activation. *Pharmacology and Therapeutics Journal*, *55*, 183–194.

Cerolini, S., Zaniboni, L., Maldjian, A., & Gliozzi, T. (2006). Effect of decosahexaenoic acid and alpha tocopherol enrichment in chicken sperm on semen quality, sperm lipid composition and susceptibility to peroxidation. *Theriogenol*, *66*, 877–886.

Chaijan, M. (2008). Lipid and myoglobin oxidations in muscle foods: A Review. *Songklanakarin Journal of Science and Technology*, *30*(1), 47–53.

Chen, B. H., & Chen, Y. C. (2001). Formation of Polycyclic Aromatic Hydrocarbons in the Smoke from Heated Model Lipids and Food Lipids. *Journal of Agricultural and Food Chemistry*, *49*(11), 5238–5243.

Chen, B. H., & Lin, Y. S. (1997). Formation of Polycyclic Aromatic Hydrocarbons during Processing of Duck Meat. *Journal of Agricultural and Food Chemistry*, *45*(4), 1394–1403.

Chen, B. H., Wang, C. Y., & Chiu, C. P. (1996). Evaluation of analysis of polycyclic aromatic hydrocarbons in meat products by liquid chromatography. *Journal of Agricultural and Food Chemistry*, *44*, 2244–2251.

Chen, C. P., Chiu, Y. S., & Lin, H. B. (1997). Comparison of GC-MS and HPLC for Overcoming Matrix Interferences in the Analysis of PAHs in Smoked Food. *Chromatographia*, *44*(9), 497–504.

Chen, X., Tume, R. K., Xu, X., & Zhou, G. (2015). Solubilization of myofibrillar proteins in water or low ionic strength media: classical techniques, basic principles and novel functionalities. *Critical Reviews in Food Science and Nutrition*. Retrieved from http://dx.doi.org/10.1080/10408398.2015.%0A1110111

Cheng, Q., & Sun, D. W. (2008). Factors affecting the water holding capacity of red meat products: A Review of Recent Research Advances. *Critical Reviews in Food Science and*

Nutrition, *48*(2), 137–159.

Chizzolini, R., Zanardi, E., Dorigoni, V., & Ghidini, S. (1999). Calorific value and cholesterol content of normal and low-fat meat and meat products. *Trends in Food Science & Technology*, *10*, 119–128.

Chow, C. K. (1975). Distribution of tocopherols in human plasma and red blood cells. *American Journal of Clinical Nutrition*, *28*, 756–760.

Chuan, B. S. (2008). *Biochemistry of triglycerides In: Principles of Biochemistry and Biophysics*. Retrieved from https://books.google.co.ug/books?isbn=8131803228

Chung, S. Y., Ramesh, R. Y., Kim, J. S., Kwon, K., Kim, M. C., & Min, B. D. (2011). Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. *Joornal of Food Chemistry*, *129*, 1420–1426.

Clark, K. A., McElhinny, A. S., Beckerle, M. C., & Gregorio, C. C. (2002). Striated muscle cytoarchitecture: An intrincate web of form and function. *Annual Review of Cell Development and Biology*, *18*, 637–706.

Claus, J. R. (2001). *Color Changes in Cooked Beef*. Madison.

Cleaves, J., & Chen, L. (2006). Experimentally tracing the key steps in the origin of Life: The Aromatic World. *Astrobiology*, *6*(3), 490–520.

Codex Alimentarius. (2005). *Code of hygiene practices for meat*.

Cooper, G. M. (2000). *The cytoskeleton and cell movement. In: the cell. A molecular Approach*. (G. M. Cooper, Ed.). ASM Press. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK9961/

Cornforth, D., & Hunt, M. (2008). *Low-oxygen packaging of fresh meat with carbon monoxide: meat quality, microbiology and safety* (AMSA White Paper Series No. 2). Savoy.

Cortinas, L., Barroeta, A., Villaverde, C., Galobart, J., Guardiola, F., & Baucells, M. D. (2005). Influence of the dietary polyunsaturation level on chicken meat quality: Lipidoxidation. *Poultry Science*, *84*, 48–55.

Creech, B. G., Feldman, G. L., Ferguson, T. M., Reid, B. L., & Couch, J. R. (1957). Exudative Diathesis and Vitamin E Deficiency in Turkey. *The Journal of Nutrition*, *62*(1), 83–95.

Da Silva, M. V. (2013). *Marketing. In: Poultry Development Review*. Rome.

Dąbrowska, M., Zielińska, A., & Nowak, I. (2015). Lipid oxidation products as a potential health and analytical problem. *Chemik*, *2*, 69–94.

Dam, H., & Glavindi, J. (1938). Alimentary exudative diathesis. *Nature*, *142*(1077).

Damaziak, K., Pietrzak, D., Michalczuk, M., Mroczek, J., & Niemiec, J. (2013). Effect of genotype and sex on selected quality attributes of turkey meat. *Archiv Für Geflügelkunde*, *77*(3), 206–214.

Damisa, M. A., & Hassan, M. B. (2009). Analysis of Factors Influencing the Consumption of Poultry Meat in the Zaria Emirate of Kaduna State, Nigeria. *European Journal of Educational Studies*, *1*(1), 1–5.

Danijela, R.-M., Vesna, V., Marija, T., Ivana, K., & Marija, G. (2013). Polyunsaturated fatty acids in health and disease. *Journal of the Serbian Chemical Society*, *78*(9), 1269–1289.

Das, U. (2006). Essential fatty acids – a review. *Current Pharmaceutical Biotechnology*, *7*, 467–482.

De Fremery, R., & Pool, M. F. (1960). Biochemistry of chicken muscle as related to rigor mortis and tenderization. *Food Research*, *25*, 73–87.

de Oliveira, J., Avanco, S. V, Garcia-Neto, M., & EPonsano, H. G. (2016). Composition of broilers meat. *Journal of Applied Poultry Research*, *25*, 173–181.

Debelle, L., & Alix, A. J. E. (1999). The structures of elastins and their function. *Biochimie*, *81*, 981–994.

Decker, E. A. (2001). *The Role of Histidine-Containing Compounds on the Buffering Capacity of Muscle: Proceedings of the 54th Reciprocal Meat Conference*. Massachusetts.

Decker, E. A. (2002). *Antioxidant mechanisms. In Food Lipids: Chemistry, Nutrition and Biotechnology*. (C. C. Akoh & D. B. Min, Eds.) (2nd ed.). New York: Marcel Dekker.

del Rio, L., Corpas, F. J., Sandalio, L. M., Palma, J. M., Gomez, M., & Barroso, J. B. (2002). Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. *Journal of Experimental Botany*, *53*, 1255–1272.

Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A. M., Carrete, J., & Pensel, N. (2007). Antioxidant status and odour profile in fresh beef frompasture or grain-fed cattle. *Meat Science*, *75*(2), 299–307.

Descalzo, A. M., & Sancho, A. M. (2008). A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Science*, *79*, 423–436.

Deshpande, S. S. (2002). *A Handbook of Food Toxicology*. New York and Basel: Mercel Mekker.

Dianzani, M., & Barrera, G. (2008). *Pathology and physiology of lipid peroxidation and its carbonyl products. In: Álvarez, S.; Evelson, P. (ed.), Free Radical Pathophysiology*. (S.

Álvarez & P. Evelson, Eds.). Kerala, India: Transworld Research Network.

Dias, J. R. (1987). Thermochim. *Acta*, *122*(2), 313.

Dias, J. R. (1988). *Handbook of Polycyclic Hydrocarbons*. Amsterdam: Elsevier.

Dinh, T. T. N., Thompson, L. D., Galyean, M. L., Brooks, J. C., Patterson, K. Y., & Boylan, L. M. (2011). Cholesterol Content and Methods for Cholesterol Determination in Meat and Poultry. *Comprehensive Reviews in Food Science and Food Safety*, *10*(1990), 269–289.

Dipple, A. (1985). *Polycyclic Aromatic Hydrocarbon Carcinogenesis. In Polycyclic Hydrocarbons and Carcinogenesis*. Washington, D.C.: American Chemical Society.

Duclos, M. J., Berri, C., & Le Bihan-Duval, E. (2007). Muscle growth and meat quality. *Journal of Applied Poultry Research*, *16*, 107–112.

Dungel, N. (1961). The special problem of cancer in Iceland with particular reference to dietary factors. *Journal of American Medical Association*, *178*, 93–95.

Edwards, S. C., Jedrychowski, W., Butscher, M., Camann, D., Kieltyka, A., & Mroz, E. (2010). Prenatal exposure to airborne polycyclic aromatic hydrocarbons and children's intelligence at 5 years of age in a prospective cohort study in Poland. *Environmental Health Perspectives*, *118*(9), 1326–1331.

EFSA. (2008). Scientific opinion of the Panel on contaminants in the food chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. *The EFSA Journal*, *724*, 65–114.

EL-Bardy, N. (2010). Cooking methods and food additives on formation of PAHs in chinken meat. *World Applied Sciences Journal*, *9*(9), 963–974.

El-Saeid, M. H. (2010). Levels of class 2A and 2B polynuclear hydrocarbons in different barbecued meat by microwave assisted extraction coupled with gas chromatography/mass spectrometry. *American-Eurasian Journal Agriculture and Environmental Sciences*, *8*, 305–312. Retrieved from http://www.idosi.org/aejaes/jaes8(3)/10.pdf

Elder, S. J., Haytowitz, D. B., Howe, J., Peterson, J. W., & Booth, S. L. (2006). Vitamin K Contents of Meat, Dairy, and Fast Food in the U.S. Diet. *Journal of Agricultural and Food Chemistry*, 463–467.

Emerson, O. H., Emerson, G. A., Mahammad, A., & Evans, H. M. (1937). The chemistry of vitamin E: tocopherols from various sources. *Journal of Biological Chemistry*, *122*, 99–107.

Endo, Y. (2018). Analytical Methods to Evaluate the Quality of Edible Fats and Oils : The

JOCS Standard Methods for Analysis of Fats , Oils and Related Materials and Advanced Methods. *Journal of Oleo Science*, *10*(1), 1–10.

Erdtsieck, B. (1989). *Quality requirements in the modern poultry industry. In Processing of Poultry*. (G. C. Mead, Ed.). New York: Elsevier Applied Science.

Erf, G. F., Bottje, W. G., Bersi, T. K., Headrick, M. D., & Fritts, C. A. (1998). Effects of Dietary vitamin E on the immune system in broilers: altered proportions of CD4 T cells in the thymus and spleen. *Poultry Science*, *77*, 529–537.

Etherington, D. J., & Sims, T. J. (1981). Detection and estimation of collagen. *Journal of the Science of Food and Agriculture*, *32*, 539–546.

EURACHEM. (1998). *The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics*. Middlesex.

Evans, H. M., Emerson, O. H., & Emerson, G. A. (1936). The isolation from wheat germ oil of an alcohol, a-tocopherol, having the properties of vitamin E. *Journal of Biological Chemistry*, *113*(1), 319–332.

FAO–WHO. (1991). *Protein Quality Evaluation*. Rome.

FAO/W H O. (2008). *Interim Summary of Conclusions and Dietary Recommendations on Total Fat & Fatty Acids*. Geneva.

FAO. (2007). *Meat processing technology for small to medium-scale producers*. Rome.

FAO. (2014a). *Food Outlook. Biannual report on global food markets.* Rome.

FAO. (2014b). *Food Outlook. Biannual report on global food markets*.

FAO. (2018a). *Gateway to poultry production and products*. Rome. Retrieved from http://www.fao.org/poultry-production-products/production/en/

FAO. (2018b). *Meat Market review*. Rome.

FAOSTAT. (2016). *FAO statistical database*. Rome.

Farmer, E. E., Krischke, M., Mueller, M. J., Sattler, S. E., Me, L., & Dellapenna, D. (2006). Nonenzymatic Lipid Peroxidation Reprograms Gene Expression and Activates Defense Markers in Arabidopsis Tocopherol-Deficient Mutants. *The Plant Cell*, *18*(December), 3706–3720.

Farombi, E. O. (2004). Diet-related cancer and prevention using anticarcinogens. *African Journal of Biotechnology*, *3*(12), 651–661.

Farwer, S. R., Boer, B. C. J. Der, Haddeman, E., Kivitis, G. A. A., Wiersma, A., & Berry, H. J. C. D. (1994). The vitamin E nutritional status of rats fed on diets high in fish oil , linseed oil or sunflower seed oil. *British Journal of Nutrition*, (May), 127–145.

Fatemi, S. H., & Hammond, G. E. (1980). Analysis of Oleate, Linoleate and Linolenate

Hydroperoxides in Oxidized Ester Mixtures. *Lipids*, *75*(5), 379—385.

Fatufe, A. A., Timmler, R., & Rodehutscord, M. (2004). Response to lysine intake in composition of body weight gain and efficiency of lysine utilization of growing male chickens from two genotypes. *Poultry Science*, *83*, 1314–1324.

Fei, J. I. (2004). *Amino acid nutrition and ideal protein for reproductive sows*. Texas Tech University.

Fennema, O. R. (1996). *Food Chemistry*. (O. R. Fennema, Ed.) (3rd ed.). New York: Marcel Dekker Inc.

Fernholz, E. (1938). On the constitution of alpha-tocopherol. *Journal of American Chemical Society*, *60*, 700–705.

Fetzer, J. C. (2000). *The Chemistry and Analysis of the Large Polycyclic Aromatic Hydrocarbons.* New York: Wiley.

Fischer, C., Hofmann, J., & Hamm, R. (1976). Erfahrungen mit der Kapillarvolumetermethode nach Hofmann zur Bestimmung des Wasserbindungsvermögens von Fleisch. *Fleischwirtschaft*, *56*, 91–95.

Fletcher, D. L. (1999). Broiler Breast Meat Color Variation, pH, and Texture. *Poultry Science*, *78*, 1323–1327.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Food and Nutrition Board. (2000). *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academy Press:

Fors, S. (1983). *Sensory properties of volatile Maillard reaction products and related compounds. In: The Maillard Reaction in Foods and Nutrition*. (G. R. Waller & M. S. Feather, Eds.). Washington: American Chemical Society.

Fouad, A. M., & El-Senousey, H. K. (2014). Nutritional factors affecting abdominal fat deposition in poultry: a review. *Asian-Australasian Journal of Animal Sciences*, *27*(7), 1057–1068.

FSAI. (2009). Polycyclic Aromatic hydrocarbons (PAHs) in Food. Retrieved January 30, 2019, from http://www.fsai.ie/surveillance/food_safety/chemical/PAH_levels.pdf

Gagné, A., Wei, S. Q., Fraser, W. D., & Julien, P. (2009). Absorption, Transport, and Bioavailability of Vitamin E and its Role in Pregnant Women. *Journal of Obstetrics and Gynaecology Canada*, *31*(3), 210–217.

Galli, F., Azzi, A., Birringer, M., Cook-Mills, J. M., Eggersdorfer, M., Frank, J., & Ozer, N.

K. (2017). Vitamin E: Emerging aspects and new directions. *Free Radical Biology and Medicine*, *102*, 16–36.

Gao, Y. T., Blot, W. J., & Zheng, W. (1987). Lung cancer among Chinese women. *International Journal of Cancer*, *40*, 604–609.

Gebauer, S., Psota, T., Harris, W., & Kris-Etherton, P. (2006). n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *American Journal of Clinical Nutrition*, *83(suppl)*, 1526S–1535S.

Gelse, K., Po, E., & Aigner, T. (2003). Collagens — structure , function , and biosynthesis. *Advanced Drug Delivery Review*, *55*, 1531–1546.

GLEAM 2. (2016). *Global Livestock Environmental Assessment Model*. Rome.

Gogus, U., & Smith, C. (2010). n-3 Omega fatty acids: a review of current knowledge. *International Journal of Food Science and Technology*, 417–436.

Gore, A. B., & Qureshi, M. A. (1997). Enhancement of humoral and cellular immunity by vitamin E after embryonic administration. *Poultry Science*, *76*, 984–991.

Gornowicz, E., Lewko, L., Pietrzak, M., & J. Gronowicz. (2009). The effect of broiler chicken origin on carcass and muscle yield and quality. *Journal of Central European Agriculture*, *109*, 193–200.

Grau, A., & Ortiz, A. (1998). Dissimilar protection of tocopherol isomers against membrane hydrolysis by phospholipase A2. *Chemistry and Physics of Lipids*, *91*(2), 109–118.

Grau, R., & Hamm, G. (1953). Eine einfache Methode zur Bestimmung der Wasserbindung im Muskel. *Die Naturwissenschaften*, *40*(1), 29–30.

Gregory, N. G. (1998). *Animal Welfare and Meat Science*. New York: CABI Publishing.

Grimmer, G., Brume, H., Dettbarn, G., Naujack, K. W., Mohr, U., & Hartung, R. W. (1988). Urinary and faecal excretion of chrysene and chrysene metabolites by rats after oral, intraperitorneal, intratracheal or intrapulmonary application. *Archives of Toxicology*, *62*, 401–405.

Grosch, W. (2014). Amino Acids , Peptides , Proteins. *Food Chemistry*, (May). https://doi.org/10.1007/978-3-540-69934-7

Grova, N., Rychen, G., Monteau, F., Le Bizec, B., & Feidt, C. (2006). Effects of oral exposure to polycyclic aromatic hydrocarbons on goat's milk contamination. *Agronomy for Sustainable Development*, *26*, 195–199.

Guardia, S., Lessire, M., Corniaux, A., Metayer-Coustard, S Mercerand, F., Tesseraud, S., Bouvarel, I., & Berri, C. (2014). Short-term nutritional strategies before slaughter are effective in modulating the final pH and color of broiler breast meat. *Poultry Science*, *93*,

1764–1773.

Guo, L., Sun, B., Shang, Z., Leng, L., Wang, Y., Wang, N., & Li, H. (2011). Comparison of adipose tissue cellularity in chicken lines divergently selected for fatness. *Poultry Science*, *90*, 2024–2034.

Hall, M., & Grover, P. L. (1990). *Polycyclic aromatic hydrocarbons: metabolisim, activation and tumor initiation*. (M. Hall & P. L. Grover, Eds.). Heidelberg: Springer- Verlag.

Halliwell, B., & Gutteridge, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, *218*, 0264-6021.

Hamm, D. (1981). Amino acid composition of breast and thigh meat from broilers produced in four locations of the United States. *Journal of Food Sciences*, *46*(4), 1122–1124.

Hamm, R. (1986). *Functional properties of the myofibrillar system and their measurements. In: Muscle as Food*. (P. J. Bechtel, Ed.). New York: Academic Press.

Hamzawy, A. H., Khorshid, M., Elmarsafy, A. M., & Souaya, E. R. (2016). Estimated Daily Intake and Health Risk of Polycyclic Aromatic Hydrocarbon by Consumption of Grilled Meat and Chicken in Egypt. *International Journal of Current Microbiology and Applied Sciences*, *5*(2), 435–448. https://doi.org/http://dx.doi.org/10.20546/ijcmas.2016.502.049

Harris, P. L., Quaife, M. L., & Swanson, W. J. (1950). Vitamin E content of foods. *Journal of Nutrition*, *40*, 367–381.

Harvey, D. (2000). *Modern Analytical Chemistry* (1st ed.). Boston: Library of Congress Cataloging-in-Publication Data.

Harvey, G. (1997). *Polycyclic Aromatic Hydrocarbons.* New York: Wiley-VCH.

Haslinger, M., Leitgeb, R., Bauer, F., Ettle, T., & Windisch, W. M. (2007). Slaughter yield and meat quality of chicken at different length of preslaughter feed withdrawal. *Die Bodenkultur*, *58*(1–4), 67–72.

Hasty, J. L., van Heugten, E., See, M. T., & Larick, D. K. (2002). Effect of vitamin E on improving fresh pork quality in Berkshire- and Hampshire-sired pigs. *Journal of Animal Science*, *80*(12), 3230–3237.

Hattemer-Frey, H. A., & Travis, C. C. (1991). Benzo-a-pyrene: environmental partitioning and human exposure. *Toxicology and Industrial Health*, *3*(7), 141–157.

Haynes, B. S. (1991). *Fossil Fuel Combustion: a Source Book*. (B. S. Haynes, Ed.). New York: Wiley.

Heinze, P. H. (2016). Electrical stimulation and meat quality. In Irene (Ed.), *Proceedings of the 6th Meat Symposium: The Meat Animal and its Products*. South Africa: ADSRI.

Herrera, E., & Barbas, C. (2001). Vitamin E: action, metabolism and perspectives. *Journal of*

Physiology and Biochemistry, *57*(1), 43–56.

Herzig, I., Navrátilová, M., Totušek, J., Suchý, P., Večerek, V., Blahová, J., & Zralý, Z. (2009). The effect of humic acid on zinc accumulation in chicken broiler tissues. *Czech Journal of Animal Science*, *54*(3), 121–127.

Honikel, K. (2004). Conversion of muscle to meat. In *Encyclopedia of meat sciences*. Elsevier Academic Press.

Honikel, K., Kim, C., & Hamm, R. (1986). Sarcomere shortening of prerigor muscles and its influence on drip loss. *Meat Science*, *16*, 267–282.

Honikel, K. O. (2009). *Moisture and water-holding capacity. In: Handbook of Muscle Foods Analysis*. (L. M. L. Nollet & F. Toldra, Eds.). Florida: CRC Press.

Honikel, K. O., & Hamm, R. (1987). Critical evaluation of methods detecting effects of processing on meat protein characteristics. In: Chemical Changes During Food Processing, Consejo Superior de Investigation. In S. Bermell (Ed.), *Proceedings of the IUFoST Symposium*.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science* (Vol. 71).

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*, *98*, 520–532.

HunterLab. (2012). Measuring Color using Hunter L, a, b versus CIE 1976 L*a*b*. Retrieved December 7, 2018, from file:///C:/Users/user/Downloads/an-1005-de.pdf

Husain, A., Naeemi, E., Dashti, B., Al-Omirah, H., & Al-Zenki, S. (1997). Polycyclic aromatic hydrocarbons in food products originating from locally reared animals in Kuwait. *Food Additives and Contaminants*, *14*, 295–299.

Hutten, G. H. P. (2000). *Semiconducting Polymers*. Weiheim: Wiley-VCH.

IARC. (2010). Overall Evaluation of Carcinogencity: [Electronic Version]. An Updating of IARC Monographs., Volumes 1 to 100.

ICH-Q2B. (1996). Validation of Analytical procedure: Methodology. In *International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use*.

ISO. (1992). *Standard 5492: terms relating to sensory analysis.*

IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). (1982). Nomenclature of tocopherols and related compounds: recommendations 1981. *European Journal*

Biochemistry, 473–475.

Jägerstad, M., & Skog, K. (2005). Genotoxicity of heat-processed foods. *Mutation Research*, *574*(1–2), 156–172.

Janoszka, B., Warzecha, L., B, U., & Bodzek, D. (2004). Organic Compounds formed in thermally treated high-protein food part I: Polycyclic Aromatic Hydrocarbons. *Acta Chromatographica*, (14), 115–128.

Jayasena, D. D., Ahn, D. U., Nam, K. C., & Jo, C. (2018). Flavour Chemistry of Chicken Meat : A Review. *Asian- Australas Journal of Animal Science*, *26*(5), 732–742.

Jedrychowski, W. A., Perera, F. P., Tang, D., Rauh, V., Majewska, R., & Mroz, E. (2013). The relationship between prenatal exposure to airborne polycyclic aromatic hydrocarbons (PAHs) and PAH-DNA adducts in cord blood. *Journal of Exposure Science and Environmental Epidemiology*, *23*, 371–377.

Jenke, D. (1996). Chromatographic method validation: a review of current practices and procedures. II. Guidelines for primary validation parameters. *Journal of Liquid Chromatography and Related Technologies*, *19*, 737–757.

Jenkins, M. Y., & Mitchell, G. V. (1989). Nutritional assessment of twelve protein foods' ingredients. *Nutrition Research*, *9*, 83–92.

Jira, W., Ziegenhals, K., & Speer, K. (2008). Gas chromatography-mass spectrometry (GC-MS) method for the determination of 16 European priority polycyclic aromatic hydrocarbons in smoked meat products and edible oils. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, *25*(6), 704–713.

Jochen, W. (2009). August Wilhelm von Hofmann (1818-1892). *Journal of Scientific Instruments*, *9*(24), 249–250.

Joshi, A. D., Kim, A., Lewinger, J. P., Ulrich, C. M., Potter, J. D., Cotterchio, M., & Stern, M. C. (2015). Cancer Medicine. *Cancer Medicine*, *4*(6), 936–952.

Josling, T., Orden, D., & Roberts, D. (2001). *Technical barriers in the global poultry: A search for "Missing Trade". Annual meeting of the International Agricultural Trade Research Consortium (IATRC),*. Auckland, New Zealand.

Juárez, M., Dugan, M. E., Aalhus, J. L., Aldai, N., Basarab, J. A., Baron, V. S., & McAllister, T. A. (2011). Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. *Meat Science*, *88*(3), 434–440.

Julie, K. N. (1997, June). Factors affecing appearance, texture and flavour. *University Bulletin 1157* .

Kamran Azad, S., Rahimi, S., & Karimi -Torshizi, M. A. (2009). Effect of dietary oil seeds on

n-3 fatty acid enrichment, performance parameters and humoral immune response of broiler chickens. *Iranian Journal of Veterinary Research*, *10*(2), 158–165.

Kanner, J., & Rosenthal, I. (1992). An assessment of lipid oxidation in foods. *Pure & Applied Chemistry*, *64*(12), 1959–1964.

Karadas, F., Erdogan, S., Kor, D., Oto, G., & Uluman, M. (2016). The effects of different types of antioxidants (Se, vitamin E and carotenoids) in broiler diets on the growth performance, skin pigmentation and liver and plasma antioxidant concentrations. *Revista Brasileira de Ciencia Avicola*, *18*(1), 101–116.

Karamouz, H., Shahryar, H. A., Ghaleh-Kandi, J. G., & Gorbani, A. (2011). Effect of inorganic chelate of zinc and restaurant residual oil added to feed mixture on the biochemical traits of thigh muscles in male broilers. *African Journal of Biotechnology*, *10*(40), 7926–7931.

Karrer, P., Frizsche, H., Ringier, B. H., & Solomon, A. (1938). Synthese des alphatocopherol. *Helvetica Chimica Acta*, *21*, 820–825.

Katalin, G. L., Tamara, B. H., & Richard, J. H. (1996). Vitamin E and vitamin C supplement use and risk of all- cause and coronary heart disease mortality in older persons : the Established Populations for Epidemiologic Studies of the. *American Journal of Clinical Nutrition*, *64*(April), 190–196. Retrieved from https://academic.oup.com/ajcn/article-abstract/64/2/190/4650406 by

Kelly, A., & Doug, C. (2014). *Nutrient Analysis Report*.

Kerr, C. L., Hammerstedt, R. H., & Barbato, G. F. (2001). Effects of selection for exponential growth rate at different ages on reproduction in chickens. *Avian Poultry Biology Reviews*, *12*(3), 127–136.

Kizi, M., Oz, F., & Besler, H. T. (2011). A Review on the Formation of Carcinogenic/Mutagenic Heterocyclic Aromatic Amines. *Food Process Technology*, *2*(5), 115–120.

Kłoczko, I. (2004). *Mięso drobiowe i jego przydatność technologiczna (eng. Poultry meat and its technological usefulness). In: Towaroznastwo żywności przetworzonej.* (F. Świderski, Ed.). SGGW, Warszawa.

Knight, P., & Parsons, N. (1988). Action of NaCl and polyphosphates in meat processing: responses of myofibrils to concentrated salt solutions. *Meat Science*, *24*, 275–300.

Kranen, R. W., Van Kuppevelt, T. H., Goedhart, H. A., Veekamp, C. H., Lambooy, E., & Veerkamp, J. H. (1999). Hemoglobin and Myoglobin Content in Muscles of Broiler Chickens. *Poultry Science*, *78*, 467–476.

Kris-Etherton, P., Harris, W., & Appel, L. (2002). *Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease.* (AHA Scientific Statement No. 106).

Ku¨hn, K. (1986). The collagen family-variations in the molecular and supermolecular structure. *Rheumatology*, *10*, 29 – 69.

Küçükyılmaz, K., Bozkurt, M., Çatlı, A. U., Herken, E. N., Çınar, M., & Bintaş, E. (2012). Chemical composition, fatty acid profile and color of broiler meat as affected by organic and conventional rearing systems. *South African Journal of Animal Science*, *42*(4), 360–368.

Kugonza, R. D., Kyarisimma, C. C., & Lisa, A. (2008). *Indigenous Chicken Flocks of Eastern Uganda, Productivity, Management and Strategies for Better Performance*.

Kurose, T., Asai, Y., Mori, E., Daitoku, D., & Kawamata, S. (2006). Distribution and change of collagen types I and III and elastin in developing leg muscle in rat. *Hiroshima Journal of Medical Sciences*, *55*(3), 85–91.

Lage, M. E., Godoy, H. T., Bolini, H. M. A., de Oliveira, R. R., & Minafra e Rezende, C S Prado, C. S. (2012). Sensory profile of warmed-over flavor in tenderloin from steers supplemented with alpha-tocopherol. *Revista Brasileira de Zootecnia*, *41*(8), 915–1920.

Lauridsen, C., Nielsen, J. H., Henckel, P., & Sorensen, M. T. (1999). Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E and copper. *Journal of Animal Science*, *77*, 105–115.

Lawless, H. (1991). The sense of smell in food quality and sensory evaluation. *Journal of Food Quality*, *14*, 33–60.

Lawrie, R. A., & Ledward, D. A. (1985). *The Eating quality of meat. In: Meat Science*. Pergamon Press.

Le Bihan-Duval, E., Millet, N., & Remignon, H. (1999). Broiler meat quality: Effect of selection for increased carcass quality and estimates of genetic parameters. *Poultry Science*, *78*, 822–826.

Leonel, F. R., Oba, A., Pelicano, E. R. L., Zeola, N. M. B. L., Boiago, M. M., Lima, T. M. A., & Souza, H. B. (2007). Performance, carcass yield, and qualitative characteristics of breast and leg muscles of broilers fed diets supplemented with vitamin E at different ages. *Brazilian Journal of Poultry Science*, *9*(2), 91–97.

Lesiak, M. T., Olson, D. G., Lesiak, C. A., & Ahn, D. U. (1996). Effects of Post-Mortem Time Before Chilling and Chilling Temperatures on Water-Holding Capacity and Texture of Turkey Breast Muscle 1 Treatments and Sample Preparation. *Poultry Science*, *76*, 552–556.

Levene, H. (1960). Robust tests for the equality of variance test for normality. In Olkin I, ed. Contributions to Probability and Statistics: In *Essays in Honor of Harold Hotelling. Palo Alto:* Stanford University Press.

Levick, S., Loch, D., Taylor, S., & Janick, J. (2007). Arachidonic acid metabolism as a potential mediator of cardiac fibrosis associated with inflammation. *Journal of Immunology*, *178*, 641–646.

Li, W. J., Zhao, G. P., Chen, J. L., Zheng, M. Q., & Wen, J. (2009). Influence of dietary vitamin E supplementation on meat quality traits and gene expression related to lipid metabolism in the Beijing-you chicken. *British Poultry Science*, *50*(2), 188–198.

Liao, G. Z., Xu, X. L., & Zhou, G. H. (2009). Effects of cooked temperatrues and addition of antioxidants on formation of heterocyclic aromatic amines in pork floss. In *Journal of Food Processing and Preservation* (Vol. 33, pp. 159–175).

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., & Bugeon, J. (2016). How muscle structure and composition influence meat and flesh quality. *Scientific World Journal*. https://doi.org/2016, 3182746.

Liu, J., Arner, A., Puolanne, E., & Ertbjerg, P. (2016). On the water-holding of myofibrils: effect of sarcoplasmic protein denaturation. *Meat Science*, *119*, 32–40.

Liu, M. B., & Liu, G. R. (2016). *Molecular Dynamics simulation of peptide – CNT interaction. In: Practicle methods for multi – scale and multi – physics*. (M. B. Liu & G. R. Liu, Eds.). World- Scientific.

Lohakare, J. D., Choi, J. Y., Kim, J. K., Yong, J. S., Shim, Y. H., Hahn, T. W., & Chae, B. J. (2005). Effects of dietary combinations of vitamin A, E and methionine on growth performance, meat quality and immunity in commercial broilers. *Asian-Australasian Journal of Animal Sciences*, *18*(4), 516–523.

Lombardi-boccia, G., & Beatriz, M. (2002). Total Heme and Non-heme Iron. *Journal of Food Science*, *67*(5), 1–5.

Lrrov, R. E., Irving, D. H., Downey, Â. E., & Tappel, A. L. (1978). Lipid peroxidation: A mechanism involved in acute ethanol toxicity as demonstrated by in vivo pentane production in the rat. *Lipids*, *13*, 305–307.

Luch, A. (2005). *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons*. London: Imperial College Press.

Luciano, F. B., Anton, A. A., & Rosa, C. F. (2007). Biochemical aspects of meat tenderness: a brief review. *Revista Archivos De Zootecnia*, *56*, 1–8.

Lukaski, H. C. (2004). Vitamin and mineral status: effects on physical performance.

Nutrition, *20*(7/8), 632–703.

Łukaszewicz, E., Kowalczyk, A., & Jerysz, A. (2016). Effect of dietary selenium and vitamin E on chemical and fatty acid composition of goose meat and liver. *Animal Science Papers and Reports*, *34*(2), 181–194.

Lynnette, R. F. (2010). Meat and cancer. *Meat Science*, *84*, 308–313.

Ma, H. (2004). Cholesterol and human health. *Nature and Science*, *2*(4), 17–21.

Machlin, L. J. (1991). *Vitamin E* (2nd ed.). New York: Marcel Dekker, Inc.

MacLeod, G. (1994). *The flavour of beef. In: Flavour of Meat and Meat Products*. (F. Shahidi, Ed.). Glasgow: Blackie Academic and Professional.

Maiorano, G., Angwech, H., Di Memmo, D., Wilkanowska, A., Mucci, R., Abiuso, C., & Tavaniello, S. (2015). Effects of intramuscular Vitamin E multiple injection on quality, oxidative stability and consumer acceptability of Laticauda lamb meat. *Small Ruminant Research*, *139*, 52–59.

Maiorano, G., Cavone, C., McCormick, R. J., Ciarlariello, A., Gambacorta, M., & Manchisi, A. (2007). The effect of dietary energy and vitamin E administration on performance and intramuscular collagen properties of lambs. *Meat Science*, *76*, 182–188.

Maiorano, G., Sobolewska, A., Cianciullo, D., Elminowska-Wenda, G., Slawinska, A., Tavaniello, S., & Bednarczyk, M. (2012). Influence of in ovo prebiotic and synbiotic administration on meat quality of broiler chickens. *Poultry Science*, *91*, 2963–2969.

Maiorano, G., Stadnicka, K., Tavaniello, S., Abiuso, C., Bogucka, J., & Bednarczyk, M. (2017). In ovo validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat. *Poultry Science*, *96*(2), 511–518.

Manahan, S. E. (1994). *Environmental Chemistry*. New York: Lewis.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat colour. *Meat Science*, *71*, 100–121.

Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., & Lucchin, L. (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. *Food and Nutrition Research*, *59*, 27606.

Marmesat, S., Morales, A., Velasco, J., & Dobarganes, M. C. (2010). Action and fate of natural and synthetic antioxidants during frying. *Grasasy Aceites*, *61*(4), 333–340.

Marsin, S. M., Susie, L. L., Zalilah, N., Wan, A., Wan, I., & Naim, A. A. (2000). Comparison of Signal-to-noise, Blank Determination, and Linear Regression Methods for the Estimation of Detection and Quantification Limits for Volatile Organic Compounds by Gas Chromatography. Retrieved from

https://pdfs.semanticscholar.org/dfaf/bd14547dc6a3f57c28b4a3c89a0aa2fb6cc8.pdf

Mašek, T., Severin, K., Gottstein, Ž., Filipović, N., Stojević, Z., & Mikulec, Ž. (2013). Effects of early castration on production performance, serum lipids, fatty acid profile and desaturation indexes in male chicken broilers fed a diet with increased fat content. *Veterinarski Arhiv*, *83*(2), 233–243.

Mason, A., Abdullah, B., Muradov, M., Korostynska, O., Al-Shamma'a, A., Bjarnadottir, S. G., & Alvseike, O. (2016). Theoretical Basis and Application for Measuring Pork Loin Drip Loss Using Microwave Spectroscopy. *Sensors*, *16*(2), 182–194.

Mattill, H. A., & Wolf, G. (2005). History of Nutrition The Discovery of the Antioxidant Function of Vitamin E : the Contribution of H. Mattill. *Journal of Nutrition*, *135*(September 2004), 363–366.

Mayer, H., Metzger, J., & Isler, O. (1967). Die stereochemic von natürlichem γ-tocotrienol (plastochromanol-3), plastochromanol-8 und plastochromemol-8'. *Helvetica Chimica Acta*, *50*, 1376–1393.

Mayne, R. (1989). Cartilage collagens—what is their function, and are they involved in articular disease? *Arthritis and Rheumatism*, *32*(3), 241 – 246.

McCormick, R. J. (1999). Extracellular modifications to muscle collagen: implications for meat quality. *Poultry Science*, *78*, 785–791.

McCormick, R. J., & Thomas, D. P. (1998). Collagen crosslinking in the heart: relationship to development and function. *Basic and Applied Myology*, *8*(2), 143–150.

Melton, S. L. (1999). *Current status of meat flavour. In: Quality Attributes of Muscle Foods*. (Y. L. Xiong, C. T. Ho, & F. Shahidi, Eds.). New York: . Kluwer Academic/ Plenum Publishers.

Menzie, C. A., Potocki, B. B., & Santodonato, J. (1992). Exposure to carcinogenic PAHs in the environment. *Environmental Science & Technology*, *26*(7), 1278–1284.

Min, B., & Ahn, D. U. (2005). Mechanism of Lipid Peroxidation in Meat and Meat Products - A Review. *Food Science and Biotechnology*, *14*(1), 152–163.

Mirghelenj, S. A., Golian, A., & Taghizadeh, V. (2009). Enrichment of chicken meat with long chain omega-3 fatty acids through diatary fish oil. *Research Journal of Biological Sciences*, *4*(5), 604–608.

Mohamed-Noor, S. E., Shuaib, Y. A., Suliman, S. E., & Abdalla, M. A. (2012). Study of microbial contamination of broilers in modern abattoirs in Khartoum State. *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology*, *36*(1), 74–80.

Mooney, J. W., Hirschler, E. M., Kennedy, A. K., Sams, A. R., & van Elswyk, M. E. (1998). Lipid and flavor quality of stored breast meat from broilers fed marine algae. *Journal of Science of Food and Agriculture*, *78*, 134–140.

Moritz, J. S., Parsons, A. S., Buchanan, N. P., Baker, N. J., Jaczynski, J., Gekara, O. J., & Bryan, W. B. (2005). Synthetic methionine and feed restriction effects on performance and meat quality of organically reared broiler chickens. *Journal of Applied Poultry Research*, *14*(3), 521–535.

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, *49*, S73–S86.

Mottet, A., & Tempio, G. (2017). L1 Global poultry production : current state and future outlook and challenges. *World's Journal of Poultry Science*, *73*(2), 245–256.

Mottier, P., Parisod, V., & Turesky, R. J. (2000). Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Barbecued Meat Sausages by Gas Chromatography Coupled to Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, *48*(4), 1160–1166.

Mottram, D. S. (1991). *Meat. In: Volatile Compounds in Foods and Beverages*. (H. Maarse, Ed.). New York: Marcel Dekker.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry*, *62*, 415–424.

Musa, H. H., Chen, G. H., Cheng, J. H., & Yousif, G. M. (2007). Relation between abdominal fat and serum cholesterol, triglycerides, and lipoprotein concentrations in chicken breeds. *Turkish Journal of Veterinary and Animal Sciences*, *31*(6), 375–379.

Mutryn, F. M., Brannick, M. E., Fu, W., Lee, R. W., & Abasht, B. (2015). Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. *BMC Genomics*, *16*, 399.

Naik, S. K., Tiwari, S. P., Sahu, T., Gendley, M. K., Dutta, G. K., & Gilhare, V. R. (2015). Effect of Organic Selenium and Vitamin E Supplementation on Physico-chemical Characteristics of Broiler Meat. *Journal of Animal Research*, *5*(3), 617–621.

Narrod, C. A., & Tiongco, M. (2012). *Global poultry sector trends and external drivers of structural change*. Rome.

Nasr, J., & Kheiri, F. (2012). Effects of lysine levels of diets formulated based on total or digestible amino acids on broiler carcass composition. *Brazilian Journal of Poultry Science*, *14*(4), 233–304.

National Institute of Medicine. (2002). *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, (fat and fatty acids).*

Washington, DC.

Neto, O. R. M., Ladeira, M. M., Chizzotti, M. L., Jorge, A. M., de Oliveira, D. M., de Carvalho, J. R. R., & do Sacramento, R. J. (2012). Performance, carcass traits, meat quality and economic analysis of feedlot of young bulls fed oilseeds with and without supplementation of vitamin E. *Revista Brasileira de Zootecnia*, *41*(7), 1756–1763.

Nicita, A. (2008). *Avian influenza and the poultry trade* (Policy research working paper No. March 2008). Washington, DC.

Niculita, P., Popa, M. E., Ghidurus, M., & Turtoi, M. (2007). Effect of vitamin E in swine diet on animal growth performance and meat quality parameters. *Polish Journal of Food and Nutrition Sciences*, *57*(1), 125–130.

O'Grady, M. N., Monahan, F. J., Fallon, R. J., & Allen, R. (2001). Effects of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. *Journal of Animal Science*, *79*, 2827–2834.

OECD/FAO. (2016). *OECD-FAO Agricultural Outlook 2016-2025*. Paris. Retrieved from http://dx.doi.org/10.1787/agr_outlook-2016-en

OECD/FAO. (2017). *OECD-FAO Agricultural Outlook*. https://doi.org/doi: dx.doi.org/10.1787/agr-outl-data-en

OECD/FAO. (2018). *OECD-FAO Agricultural Outlook 2018-2027*. Paris/Rome. Retrieved from https://doi.org/10.1787/agr_outlook-2018-en

Offer, G., & Cousins, T. (1992). The mechanism of drip production – formation of 2 compartments of extracellular-space in muscle postmortem. *Journal of the Science of Food and Agriculture*, *58*, 107–116.

Offer, G., & Knight, P. (1988). *The structural basis of water-holding in meat. Part 1: general principles and water uptake in processing: In Developments in Meat Science*. (R. A. Lawrie, Ed.) (4th ed.). London: Elsevier Science.

Offer, G., & Trinick, J. (1983). On the mechanism of water-holding in meat: the swelling and shrinking of myofibrils. *Meat Science*, *8*, 245–281.

Ohvo-Rekilä, H., Ramstedt, B., Leppimäki, P., & Slotte, J. P. (2002). Cholesterol interactions with phospholipids in membranes. *Progress in Lipid Research*, *41*, 66–97.

Ojok, L. (1993). Diseases as an important factor affecting increased poultry production in Uganda. *Jahnguan*, *94*, 37–44.

Olivier, M., Hollstein, M., & Hainaut, P. (2010). TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harbor Perspectives in Biology*. https://doi.org/10.1101/cshperspect.a001008

Olson, R. E. (1998). Discovery of the lipoproteins, their role in fat transport and their significance as risk factors. *Journal of Nutrition*, *128*(128), 439S–443.

Owens, C. M., Cavitt, L. C., & Meullenet, J. . (2004). Tenderness Evaluation in Poultry Meat. In *Proceedings of the 57th American Meat Science Association Reciprocal Meat Conference June 20-23, 2004*, (pp. 115–121). Kentucky.

Packer, L., Weber, S. U., & Rimbach, G. (2001). Molecular aspects of α-tocotrienol antioxidant action and cell signalling. *Annals of Biological Research.The Journal of Nutrition*, *131*(2), 131.

Panickar, K. S., & Bhathena, J. S. (2010). *Control of fatty acid intake and the role of essential fatty acids in cognitive function and neurological disorders. In: Fat Detection: Taste, Texture, and Post Ingestive Effects*. (J. P. EMontmayeur & le Coutre J., Eds.). Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK53554/

Pappeniieimear, M., & Goerncir, M. (1931). Cerebellar disorder in chicks, apparently of nutritional origin. *Journal of Experimental Medicine*, *53*, 11–26.

Pasca, I., Oroian, T., Oroian, R., Pusta, D., Morar, R., Pascalau, S., & Bagita, C. (2008). The phenotipical performances of Ross 308 and Cobb 500, exploited in the North Western part of Romania. *Bulletin USAMV Cluj-Napoca, Veterinary Medicine*, *65*(1), 340–345.

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh quality attributes: a review. *Meat Science*, *89*, 111–124.

Pearson, A. M., & Gillett, T. A. (1996). *Composition and nutritive value of raw materials and processed meat. In: Processed meat*. (A. M. Pearson & T. A. Gillett, Eds.). Springer-Scence+Business Media.

Penning, T. M., Ohnishi, S. T., Ohnishi, T., & Harvey, R. G. (1996). Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon dihydrodiols catalyzed by dihydrodiol dehydrogenase. *Chemical Research InToxicology*, *9*, 84–92.

Pennock, J. F., Hemming, F. W., & Kerr, J. D. (1964). Reassessment of tocopherol chemistry. *Biochemical and Biophysical Research Communications*, *17*, 542–548.

Perez, G., Lilla, E., & Cristalli, A. (1986). Formation of polycyclic aromatic hydrocarbons by ionizing radiations. *Chemosphere*, *15*, 589–594.

Perić, L., Milošević, N., Žikić, D., Kanački, Z., Džinić, N., Nollet, L., & Spring, P. (2009). Effect of selenium sources on performance and meat characteristics of broiler chickens. *Journal of Applied Poultry Research*, *18*(3), 403–409.

Pesti, M. G. (2009). Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. *Journal of Applied Poultry Research*, *18*, 477–486.

Peter, R. S. M. (1977). *Principles of Organic Chemistry* (2nd ed.). Oxford: Heinmann Educationa Books.

Petracci, M., Mudalal, S., Soglia, F., & Cavani, C. (2015). Meat quality in fast-growing broiler chickens. *World's Poultry Science Journal*, *71*, 363–373.

Pilarski, R., Bednarczyk, M., Lisowski, M., Rutkowski, A., Bernacki, Z., Wardeńska, M., & Gulewicz, K. (2005). Assessment of the effect of α-galactosides injected during embryogenesis on selected chicken traits. *Folia Biologica*, *53*(1–2), 13–20.

Pompeu, M. A., Cavalcanti, L. F. L., & Toral, F. L. B. (2018). Effect of vitamin E supplementation on growth performance, meat quality, and immune response of male broiler chickens: A meta-analysis. *Livestock Science*, *208*(March 2017), 5–13.

Ponte, P. I. P., Prates, J. A. M., Crespo, J. P., Crespo, D. G., Alves, S. P., Ferreira, L. M. A., & Fontes, C. M. G. A. (2008). Improving the lipid nutritive value of poultry meat through the incorporation of a dehydrated leguminous-based forage in the diet for broiler chicks. *Poultry Science*, *87*, 1587–1594.

Pospiech, E., Grześ, B., Mikołajczak, B., Iwańska, E., & Łyczyński, A. (2007). Proteins of meat as a potential indicators its quality- a review. *Polish Journal of Food and Nutrition Sciences*, *57*(1), 11–16.

Pospiech, E., & Montowska, M. (2011). *Technologies to improve water-holding capacity of meat*. (S. T. Joo, Ed.). Kerala: Research Signposts.

Puisiuex, A., Lim, S., Groupman, J., & Ozturk, M. (1991). Selective targetting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. *Cancer Research*, *51*, 6185–6189.

Puolanne, E., & Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science*, *86*(1), 1–15.

Puolanne, E. J., Pösö, A. R., Ruusunen, M. H., Sepponen, K. V, & Kylä-puhju, M. S. (2002). Lactic Acid in Muscle and its Effects on Meat Quality. In *Proceedings of the 55th Reciprocal Meat Conference* (pp. 57–62). Helsinki.

Qiu, X. (2003). Biosynthesis of docosahexaenoic acid (DHA, 22:6-4,7,10,13,16,19): Two distinct pathways. *Prostaglandins Leukotrienes Essential Fatty Acids*, *68*, 181–186.

Rahman, K. (2007). Studies on free radicals, antioxidants and co-factors. *Clinical Interventions in Aging*, *2*, 219–236.

Ramesh, A., Inyang, F., Hood, D. B., Archibong, A. E., Knuckles, M. E., & Nyanda, A. M.

(2001). Metabolism, bioavaliability, and toxicokinettics of Benzo[alpha]pyrene in F-344 rats following oral administration. *Experimental and Toxicologic Pathology*, *53*(4), 275–290.

Ramesh, A., Walker, S. A., Hood, D. B., Guillén, M. D., Schneier, K., & Weyand, E. H. (2004). Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *International Journal of Toxicology*, *23*, 301–333.

Ravindra, K., Wauters, E., Taygi, S. K., Mor, S., & Van, R. (2006). Assessment of air quality after the implementation of CNG as fuel in public transport in Delhi, India. *Environmental Monitoring and Assessment*, *115*, 405–417.

Reicks, A. L. (2006). *Consumer motivations and the impact of brand on purchasing preferences of fresh beef*. Texas Tech University.

Rengaraj, D., & Hong, Y. H. (2015). Effects of dietary vitamin E on fertility functions in poultry species. *International Journal of Molecular Sciences*, *16*, 9910–9921.

Rizvi, S., Raza, S. T., Ahmed, F., Ahmad, A., Abbas, S., & Mahdi, F. (2014). The role of vitamin E in Human Health and Some Diseases. *Sultan Qaboos University Med Journal*, *14*(May), 157–165.

Robinson, J. M. (2008). Reactive oxygen species in phagocytic leukocytes. *Histochem Cell Biology*, *130*, 281–297.

Rossi, R., Pastorelli, G., Cannata, S., Tavaniello, S., Maiorano, G., & Corino, C. (2013). Effect of long term dietary supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork. *Meat Science*, *95*, 542–548.

Ruan, E. D., Juárez, M., Thacker, R., Yang, X., Dugan, M. E. R., & Aalhus, J. L. (2014). Dietary vitamin E effects on the formation of heterocyclic amines in grilled lean beef. *Meat Science*, *96*, 849–853.

Rule, D. C., Broughton, K. S., Shellito, S. M., & Maiorano, G. (2002). Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. *Journal of Animal Science*, 1202–1211.

Russo, M. V., Avino, P., & Notardonato, I. (2016). Advances in Fast analysis of nine PAHs in beer by ultrasound- trap mass spectrometry. *RSC Advances*, *6*, 13920–13927.

Rustan, A. C., & Drevon, C. A. (2005). Fatty Acids: Structures and Properties. In *Encyclopedia Of Life Sciences.* (pp. 1–7). John Wiley & Sons. Retrieved from https://www.uio.no/studier/emner/matnat/farmasi/FRM2041/v06/undervisningsmateriale/fatty_acids.pdf

Sadikovic, B., & Rodenhiser, D. I. (2006). Benzopyrene exposure disrupts DNA methylation

and growth dynamics in breast cancer cells. *Toxicology and Applied Pharmacology*, *216*(3), 458–468.

Sage, I. C. (1998). *Handbook of Liquid Crystals*. Weinheim: Wiley-VCH.

Sainsbury, M. (1992). , *Aromatic Chemistry*. London: Oxford University Press.

Salma, U., Miah, A. G., Maki, T., Nishimura, M., & Tsujii, H. (2007). Effect of dietary Rhodobacter capsulatus on cholesterol concentration and fatty acid composition in broiler meat. *Poultry Science*, *86*, 1920–1926.

Salvatori, G., Pantaleo, L., Di Cesare, C., Maiorano, G., Filetti, F., & Oriani, G. (2004). Fatty acid composition and cholesterol content of muscle as related to genotype and vitamin E treatment in crossbred lambs. *Meat Science*, *67*, 45–55.

Sampath, H., & Ntambi, J. (2004). Polyunsaturated fatty acid regulation of gene expression. *Nutrition Reviews*, *62*, 333–339.

Sams, A. R. (1998). Meat quality during processing. *Poultry Science*, *78*, 798–803.

Samuel, G., Angela, M., Bryan, G., Jill, G., David, J., & John, R. (2010). *Screen for the presence of Polycyclic aromatic hydrocarbons in select seafoods using LC-Fluorescence* (1st ed.). Minnesota: . Minnesota:Forensic Chemistry Center (FCC), Food and Drug Administration.

SCF. (2002). *Opinion of the Scientific Committee on Food (SCF) in the risks to human health of Polycyclic Aromatic Hydrocarbons in Food*. Brussels.

Schiavone, A., Marzoni, M., Castillo, A., Nery, J., & Romboli, I. (2010). Dietary lipid sources and vitamin E affect fatty acid composition or lipid stability of breast meat from Muscovy duck. *Canadian Journal of Animal Science*, *90*, 371–378.

Schmölz, L., Birringer, M., Lorkowski, S., & Wallert, M. (2016). Complexity of vitamin E metabolism. *World Journal of Biological Chemistry*, *7*, 14–43.

Schwerdtle, T., Ebert, F., Thuy, C., Richter, C., Mullenders, L. H., & Hartwig, A. (2010). Genotoxicity of soluble and particulate cadmium compounds: impact on oxidative DNA damage and nucleotide excision repair. *Chemical Research in Toxicology*, *23*(2), 432–442.

Sciiwartz, K. (1957). Prevention of exudative diathesis in chicks by factor 3 and selenium. *Proceedings of Society for Experimental Biology and Medine*, *95*, 621.

Seeds, M., & Bass, D. (1999). Regulation and metabolism of arachidonic acid. *Clinical Reviews in Allergy and Immunology*, *17*, 5–26.

Sellier, N., Arnould, C., Gue, D., Boutten, B., Jego, Y., Beaumont, C., & Bihan-duval, E. Le. (2003). Variation of Chicken Technological Meat Quality in Relation to Genotype and

Preslaughter Stress Conditions. *Poultry Science*, *82*, 1829–1838.

Seo, S. H., Lee, H. K., Lee, W. S., Shin, K. S., & Paik, I. K. (2008). The Effect of Level and Period of Fe-methionine Chelate Supplementation on the Iron Content of Boiler Meat. *Asian-Australian Journal of Animal Science*, *21*(10), 1501–1505.

Shahidi, F., & Zhong, Y. (2005). *Lipid Oxidation: measurement methods. In: Bailey's Industrial Oil and Fat Products*. (F. Shahidi, Ed.). John Wiley & Sons, Inc., Publication.

Šimek, J., Vorlová, L., Malota, L., Steinhauserová, I., & Steinhauser, L. (2003). Post-mortal changes of pH value and lactic acid content in the muscles of pigs and bulls. *Czech Journal of Animal Science*, *48*(7), 295–299.

Simko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Science*, *770*(2), 3–18.

Simopoulos, A. (2006). Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomedicine and Pharmacotherapy*, *60*, 502–507.

Singh, Z., Karthigesu, I. P., Singh, P., & Kaur, R. (2014). Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: a review. *Iranian Journal of Publick Health*, *43*(3), 7–16.

Sirri, F., Bianchi, M., Petracci, M., & Meluzzi, A. (2009). Influence of partial and complete caponization on chicken meat quality. *Poultry Science*, *88*, 1466–1473.

Sitrin, M. D., & Bengoa, J. M. (1987). Intestinal absorption of cholecalciferol and 25-hydroxycholecalciferol in chronic cholestatic liver disease. *American Journal of Clinical Nutrition*, *46*, 1011–1015.

Skřiva, M., Dlouhá, G., Englmaierová, M., & Červinková, K. (2010). Effects of different levels of dietary supplemental caprylic acid and vitamin E on performance, breast muscle vitamin E and A, and oxidative stability in broilers. *Czech Journal of Animal Science*, *55*, 167–173.

Skupinska, K., Misiewics, I., & Kasprzycka-Guttman, T. (2004). Polycyclic aromatic hydrocarbons: physicochemical properties, environmental appearance and impact on living organisms. *Acta Poloniae Pharmaceutica-Drug Research*, *61*(3), 233–240.

Smith, D. M. (2010). *Functional properties of muscle proteins in processed poultry products. In: Poultry Meat Processing*. (C. M. Owens, C. Z. Alvarado, & A. R. Sams, Eds.) (2nd editio). CRC Press, Taylor & Francis Group.

Smithgall, T. E., Harvey, R. G., & Penning, T. M. (1998). Spectroscopic identification of

ortho-quinone as the products of polycyclic aromatic trans dihydrodiol oxidation catalysed by dihydrodiol dehydrogenase: A potential route of proximate carcinogen metabolism. *Journal of Biology and Chemistry*, *263*, 1814–1820.

Sochor, J., Ruttkay-Nedecky, B., Babula, P., Adam, V., Hubalek, J., & Kizek, R. (2012). *Automation of Methods for Determination of Lipid Peroxidation. In Lipid Peroxidation*. Retrieved from http://dx.doi.org/10.5772/45945

Sorensen, G., & Jorgensen, S. S. (1996). A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. *Z Lebensm Unters Forsch*, *202*(3), 205–210.

Sorensen, P., & Ssewanyana, E. (2003). Progress of the SAARI chicken breeding project-Analysis of growth capacity. In *Proceedings of the LSRP Annual Scientific Workshop, March 2003*. Kampala.

Soriano-Santos, J. (2010). *Chemical composition and nutritional content of raw poultry meat. In: Handbook of Poultry Science and Technology, Volume 1: Primary Processing.* (I. Guerrero-Legarreta & Y. H. Hui, Eds.). John Wiley & Sons, Inc.

Soriano, J. (2018). *Chemical composition and Nutritional content of raw poultry meat*. (G.-L. Isabel & Y. H. Hui, Eds.). Iztapalapa, Mexico: John Wiley & Sons, Inc. https://doi.org/10.1002/9780470504451.ch25

Spence, C., Levitan, C. A., Shankar, M. U., & Zampini, M. (2010). Does Food Color Influence Taste and Flavor Perception in Humans? *Chemosensory Perception*, *3*(March), 68–84.

Ssewannyana, E., Ssali, A., Kasadha, T., Dhikusoka, M., Kasoma, Kwatotyo, B. A., & Aziku, L. (2003). Characterisation of indigenous chickens in Uganda Kampala. In *Proceedings of the LSRP Annual Scientific Workshop,*. Kampala.

Stadler, H. R., & Lineback, R. D. (2009). *Process Induced Food Toxicants: Occurence, Formation, Mitigation and Health Risks*. Hoboken New Jersey: John Wiley and Sons Inc.

Stern, M. G., Robeson, C. D., Weisler, L., & Baxter, J. G. (1947). δ-Tocopherol I: isolation from soybean oil and properties. *Journal of American Chemical Society*, *69*, 869–874.

Stohs, S. J., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. Free. *Radical Biology & Medicine*, *18*(2), 321–336.

Stratova, E., Suchy, P., Vitula, F., & Vecerek, V. (2006). Differences in the amino acid composition of mucles from pheasant and broiler chickens. *Arch Tierz Dummerstof*, *49*(5), 508–514.

Suchý, P., Straková, E., & Herzig, I. (2014). Selenium in poultry nutrition: a review. *Czech Journal of Animal Science*, *59*(11), 495–503.

Suematsu, T., Matsumurta, T., Santon, N., Miyamotto, T., Ooka, T., Kamada, T., & Abe, H. (1981). Lipid peroxidation in alco holic liver disease in humans. *Alcoholism (NY)*, *5*, 427–430.

Sugimura, T., Wakabayashi, K., Nakagama, H., & Nagao, M. (2004). Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. In *Cancer Science* (Vol. 95, pp. 290–299).

Suman, S. P., Mancini, R. A., & Faustman, C. (2006). Lipid-oxidation-induced carboxymyoglobin oxidation. *Journal of Agricultural and Food Chemistry*, *54*, 9248–53.

Surai, P. F., Sparks, N. H. C., & Speake, B. K. (2006). The role of antioxidants in reproduction and fertility of poultry. Retrieved December 14, 2018, from http://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-italy-2006/10907.pdf

Surendranath, P. S., & Poulson, J. (2013). Myoglobin Chemistry and Meat Color. *Annual Review of Food Science and Technology*, *4*, 79–99.

Swatland, H. J. (1994). *Physical properties of meat. In: Structure and development of meat animals and poultry*. (H. J. Swatland, Ed.). Technomic Publishing AG.

Takatsuki, K., Suzuki, S., Sato, N., & Ushizawa. (1985). Liquid chromatographic determination of polycyclic aromatic hydrocarbons in fish and shellfish. *Journal of the Association of Official Analytical Chemists*, *68*, 945–949.

Tang, J., Faustman, C., & Hoagland, T. A. (2004). Krzywicki revisited: equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. *Journal of Food Sciences*, *69*, C717–20.

Tarantini, A., Maitre, A., Lefebvre, E., Marques, M., Rajhi, A., & Douki, T. (2011). Polycyclic aromatic hydrocarbons in binary mixtures modulate the efficiency of benzo[a]pyrene to form DNA adducts in human cells. *Toxicology*, *279*, 36–44.

Tartrakoon, W., Tartrakoon, T., & Kitsupee, N. (2016). Effects of the ratio of unsaturated fatty acid to saturated fatty acid on the growth performance , carcass and meat quality of fi nishing pigs. *Animal Nutrition*, *2*(2), 79–85.

Tavaniello, S., Maiorano, G., Stadnicka, K., Mucci, R., Bogucka, J., & Bednarczyk, M. (2018). Prebiotics offered to broiler chicken exert positive effect on meat quality traits irrespective of delivery route. *Poultry Science*, *97*(8), 2979–2987.

Tavaniello, S., Mucci, R., Stadnicka, K., Acaye, O., Bednarczyk, M., & Maiorano, G. (2019).

Effect of in ovo administration of different synbiotics on carcass and meat quality traits in broiler chickens. *Poultry Science*, *98*, 464–472.

Tavasalkar, S. U., Mishra, H. N., & Madhavan, S. (2012). Evaluation of Antioxidant Efficacy of Natural Plant Extracts against Synthetic Antioxidants in Sunflower Oil. Retrieved December 12, 2018, from http://www.omicsonline.org/scientific-reports/2157-7110-SR-504.pdf

Tewari, K., Vishnoi, N., & Mehrotra, S. (1998). *A textbook of Organic Chemistry*. (K. Tewari, N. Vishnoi, & S. Mehrotra, Eds.) (2nd ed.). New Delhi: Vikas Publishing House Pte Ltd.

The American Heritage. (2009). *Dictionary of the English Language* (4th ed.). Houghton Mifflin Company.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B. Biological Sciences*, *365*, 2853–2867.

Tian, W. N., Braunstein, L. D., Pang, J., Stuhlmeier, K. M., Xi, Q. C., Tian, X., & Stanton, R. C. (1998). Importance of Glucose-6-phosphate Dehydrogenase Activity for Cell Growth. *The Journal of Biological Chemistry*, *273*(17), 10609–10617.

Tim, V., & Young McEneny, J. (2001). Lipoprotein oxidation and atherosclerosis. *Biochemical Society Transactions*, *2*, 358–362.

Tornberg, E. (2005). Effects of heat on meat proteins – Implications on structure and quality of meat products. *Meat Science*, *70*, 493–508.

Traber, M. G., & Atkinson, J. (2007). Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medicine*, *43*(1), 4–15.

Trout, G. R. (1988). Techniques for measuring water-binding capacity in muscle foods, a review of methodology. *Meat Science*, *23*, 235-252.

Trout, G., & Schmidt, G. R. (1987). The effect of cooking temperature on the functional properties of beef proteins: the role of ionic strength, pH and pyrophosphate. *Meat Science*, *20*, 129–147.

USDA. (2018a). *Livestock and Poultry: World Markets and Trade*.

USDA. (2018b). *Top 10 Foods Highest in Vitamin E*. New York. Retrieved from https://www.myfooddata.com/articles/vitamin-e-foods.php

Vahmani, P., Mapiye, C., Prieto, N., Rolland, D. C., McAllister, T. A., Aalhus, J. L., & Dugan, M. E. R. (2015). The scope for manipulating the polyunsaturated fatty acid content of beef: a review. *Journal of Animal Science and Biotechnology*, *6*(29), 1–13.

van der Rest, M. R. (1991). Garrone, Collagen family of proteins. *Federation of American Societies for Experimental Biology Journal (FASEB J)*, *5*, 2814 – 2823.

van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Nutritional value of insects for human consumption. In: Edible insects: future prospects for food and feed security*. Rome.

van Laack, R. L. J. M. (2000). Determinants of Ultimate pH of Meat and Poultry.

Van Laack, R. L., Liu, C. H., Smith, M. O., & Loveday, H. D. (2000). Characteristics of pale, soft, exudative breast meat. *Poultry Science*, *79*, 1057–1061.

Vandeplas, S., Marcq, C., Dauphin, R. D., Beckers, Y., Thonart, P., & Théwis, A. (2008). Contamination of poultry flocks by the human pathogen Campylobacter spp. and strategies to reduce its prevalence at the farm level. *Biotechnology, Agronomy, Society and Environment, 12(3),* 317-334.

Vieira, S. L., & Angel, C. R. (2012). Optimizing broiler performance using different amino acid density diets: What are the limits? *Journal of Applied Poultry Research, 21*, 149–157.

Vognarová, I., Dvorák, Z., & Böhm, R. (1996). Collagen and elastin in different cuts of veal and beef. *Journal of Food Science*, *33*(4), 339–343.

Wang, F., Sun, G. P., & Zou, Y. F. (2012). Meat consumption and risk of lung cancer: evidence from observational studies. *Annals of Oncology*, *23*(12), 3163–3170.

Wang, J., Joshi, A. D., Corral, R., Siegmund, K. D., Martinez, E., Sandler, R. S., & Stern, M. C. (2014). Carcinogen metabolism genes, red meat and poultry intake, and colorectal cancer risk. *International Journal of Cancer*, *130*(8), 1–20.

Wang, S., & Yu, H. (2005). Effects of co-Existing biologically relevant molecules and ions on DNA photocleavage caused by pyrene and its derivatives. *International Journal of Environmental Research and Public Health*, *2*(1), 132–137.

Wang, W., Yang, H., Wang, Z., Han, J., Zhang, D., Sun, H., & Zhang, F. (2015). Effects of prebiotic supplementation on growth performance, slaughter performance, growth of internal organs and small intestine and serum biochemical parameters of broilers. *Journal of Applied Animal Research*, *43*(1), 33–38.

Warner, R. (2014). Measurement of meat quality/ measurements of water-holding capacity and color: objective and subjective. In C. Devine & M. Dikeman (Eds.), *Encyclopedia of Meat Sciences,* (2nd ed.). Oxford: Academic Press.

Wąsowicz, E., Gramza, A., Hęś, M., Jeleń, H. H., Korczak, J., Samotyja, U., & Zawirska-Wojtasiak, R. (2004). Oxidation of lipids in food. *Polish Journal Food Nutrition Sciences*, *13*(54), 87–100.

Węglarz, A. (2010). Meat quality defined based on pH and color depending on cattle category

and slaughter season. *Czech Journal of Animal Science*, *55*(12), 548–556.

Whittle, K. J., Dunphy, P. J., & Pennock, J. F. (1966). The isolation and properties of δ-tocotrienol from Hevea latex. *Biochemical Journal*, *100*, 138–145.

WHO. (1987). *Polynuclear aromatic hydrocarbons (PAHs). Air Quality Guidelines for Europe105-117*. Geneva.

WHO. (1998). *Environmental Health Criteria 202, Selected Non-heterocyclic PAHs*. Geneva.

WHO. (2000). *Poyccyclic Aromatic Hydrocarbons. In Air Quality Guidelines* (2nd ed.). Copenhagen.

WHO. (2015). IARC Monographs to evaluate consumption of red meat and processed meat and cancer risk. *International Agency of Research on Cancer*, (October), 1–2. https://doi.org/http://www.who.int/features/qa/cancer-red-meat/en/

Willson, R. L. (1983). Free radical protection: why vitamin E, not vitamin C, ÃŸ-caroteneor glutathione. In R. Porter & J. Whelan (Eds.), *Biology of vitamin E* (pp. 19–44). London: Pitman Books Ltd.

Wilson, K. M., Mucci, L. A., Drake, B. F., Preston, M. A., Stampfer, M. J., Giovannucci, E., & Kibel, A. S. (2016). Meat , Fish , Poultry , and Egg Intake at Diagnosis and Risk of Prostate Cancer Progression. *Cancer Prevention Research*, *9*(12), 933–942.

Windhorst, H.-W. (2018). *Patterns of EU poultry meat production and consumption – A 2016 status report*. Vechta.

Wojcik, M., & Burzynska-Pedziwiatr, I Wozniak, L. A. (2010). A review of natural and synthetic antioxidants important for health and longevity. *Current Medicinal Chemistry*, *17*, 3262–3288.

Wolk, A. (2017). Potential health hazards of eating red meat. *Journal of Internal Medicine*, *281*(2), 106–122.

Wong, D., Cheng, K. W., & Wang, M. (2012). Inhibition of heterocyclic amine formation by water-soluble vitamins in Maillard reaction model systems and beef patties. *Food Chemistry*, *1333*, 760–766.

Wong, W. W. H. (2015). Polycyclic aromatic hydrocarbons for organic photovoltaics. In *Communications and Photonics Conference and Exhibition, 2011. ACP. Asia*. https://doi.org/10.1117/12.902704

Wood, J. D., Richardson, R. I., Nute, G. ., Fisher, A. V, Campo, M. ., Kasapidou, E., & Enser, M. (2003). Effects of fatty acids on meat quality: A review. *Meat Science*, *66*, 21–32.

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. *Amino Acids*, *37*, 1–17.

Wu, J. H., & Croft, K. D. (2007). Vitamin E metabolism. *Molecular Aspects of Medicine*, *28*,

437–452.

Wu, J. H. Y., Ward, N. C., Indrawan, A. P., Almeida, C., Hodgson, J. M., Proudfoot, J. M., & Croft, K. D. (2007). Effects of a -Tocopherol and Mixed Tocopherol Supplementation on Markers of Oxidative Stress and Inflammation in Type 2 Diabetes. *Clinical Chemistry*, *53*(3), 511–519.

Xiong, Y. L., & Decker, E. A. (1995). Alterations of muscle protein functionality by oxidative and antioxidative processes. *Journal of Muscle Foods Banner*, *6*(2), 139–160.

Xue, W., & Warshawsky, D. (2005). Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: A review. *Toxicology and Applied Pharmacology*, *206*(1), 73–93.

Yamauchi, R. (2003). Oxidation Products of Vitamin E in the Peroxidation of Liposomal and Biological Systems. *Journal of Clinical Biochemistry Nutrition*, *34*, 111–120.

Young, L. L., Cason, J. A., & Lyon, C. E. (1993). Effect of pH on color of cooked turkey meat. *Poultry Science*, *72*(Suppl. 1), 418.

Young, L. L., Lyon, C. E., Northcutt, J. K., & Dickens, J. (1996). Effect of Time Post-Mortem on Development of Pink Discoloration in Cooked Turkey Breast Meat. *Poultry Science*, *75*, 140–143.

Young, V. R., & Pellet, P. L. (1994). Plant proteins in relation to human protein and amino acid nutrition. *American Journal of Clinical Nutrition*, *59*, 1203S–1212S.

Yu, H. (2002). Environmental carcinogenic polycyclic aromatic hydrocarbons: photochemistry and phototoxicity. *Journal of Environmental Science and Health*, *20*, 149–183.

Zayas, J. F. (1997a). *Solubility of meat and fish proteins. In: Functionality of Proteins in Food*. (J. F. Zayas, Ed.). Springer.

Zayas, J. F. (1997b). *Water Holding Capacity of proteins. In: Functionality of proteins in food*. (J. F. Zayas, Ed.). Springer.

Zduńczyk, Z., & Jankowski, J. (2013). Poultry meat as functional food: modification of the fatty acid profile – a review. *Annals of Animal Cience*, *13*(3), 463–480.

Zeb, A., & Ullah, F. (2016). A Simple Spectrophotometric Method for the Determination of Thiobarbituric Acid Reactive Substances in Fried Fast Foods. *Journal of Analytical Methods in Chemistry*, *2016*.

Zhou, G. D., Richardson, M., Fazili, I. S., Wang, J., Donnelly, K. C., & Wang, F. (2010). Role of retinoic acid in the modulation of benzo (a) pyrene-DNAadducts in human hepatoma cells: implications for cancer prevention. *Toxicololgy and Applied*

Pharmacology, *249*, 224–230.

Ziaei, N., Moradikor, N., & Pour, E. E. (2013). The effects of different levels of vitamin-E and organic selenium on performance and immune response of laying hens. *African Journal of Biotechnology*, *12*(24), 3884–3890.

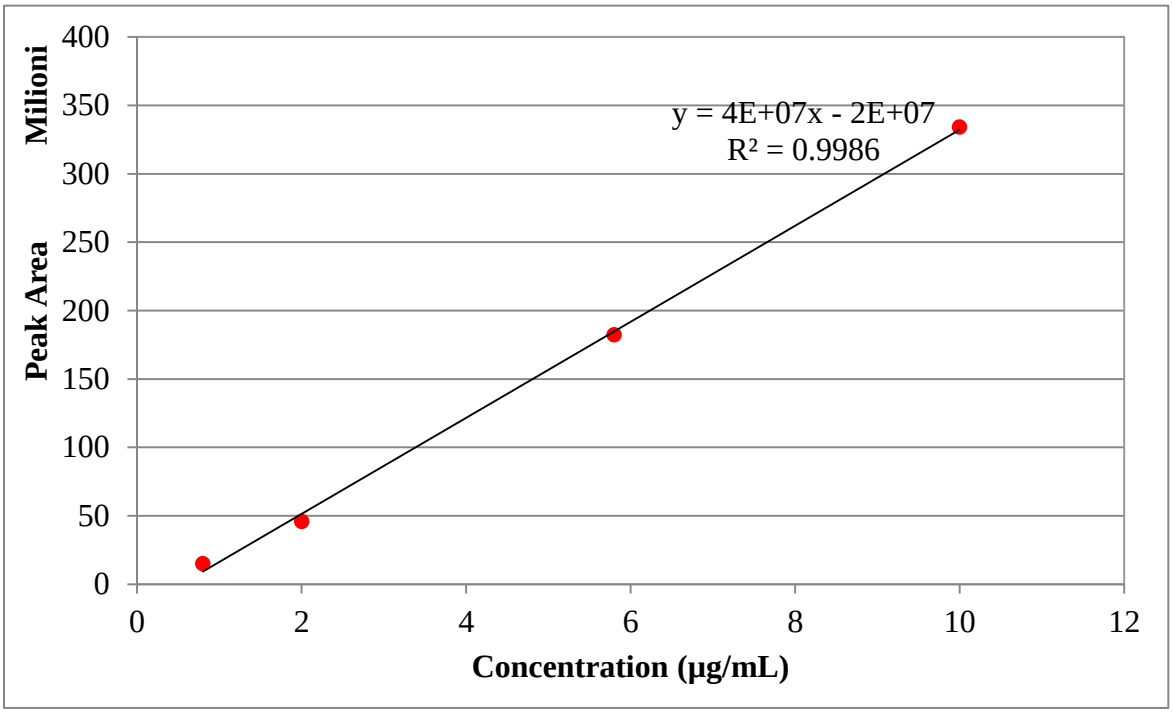
Ziech, D., Franco, R., Pappa, A., & Panayiotidis, M. I. (2011). Reactive oxygen species (ROS)-induced genetic and epigenetic alterations in human carcinogenesis. *Mutation Research*, *711*(1), 167–173.

Zielińska, A., & Nowak, I. (2014). Tocopherols and tocotrienols as vitamin E. *Chemik*, *68*(7), 585–591.

Zymon, M., Strzetelski, J., Pustkowiak, H., & Sosin, E. (2007). Effect of freezing and frozen storage on fatty acid profile of calves meat. *Polish Journal of Food and Nutrition Science*, *57*(4), 647–650.

APPENDICES

Appendix 1 Calibration curve for Benzo(b)Fluoranthene



Appendix 2 Amendment of Annex to Regulation (EC) No 1881/2006

The Annex to Regulation (EC) No 1881/2006 is amended as follows:

(1) Section 6: ***Polycyclic aromatic hydrocarbons*** is replaced by the following:

Foodstuffs		Maximum levels ***(µg/kg)***	
6.1	Benzo(a)pyrene, benzo (a) anthracene, benzo (b) fluoranthene and chrysene	Benzo(a)pyrene	Sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene[(45)]
6.1.1	Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	2.0	10.0

6.1.2	Cocoa ***beans and derived products***	5.0 µg/kg ***fat as from 1.4.2013***	35,0µg/kg ***fat as from 1.4.2013 until 31.3.2015*** ***30,0 µg/kg fat as from 1.4.2015***
6.1.3	Coconut oil intended for direct human consumption or use as an ingredient in food	2.0	20.0
6.1.4	Smoked meat and smoked meat products; ***heat treated meat*** and ***heat treated meat products*** sold to the final consumer	5,0 until 31.8.2014 2.0 as from 1.9.2014	30.0 as from 1.9.2012 until 31.8.2014 12.0 as from 1.9.2014
6.1.5	Muscle meat of smoked fish and smoked fishery products [(25)(36)], excluding fishery products listed in points 6.1.6 and 6.1.7. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen[(44).] In case of smoked crabs and crab-like crustaceans (*Brachyura* and *Anomura*) it applies to muscle meat from appendages.	5.0 until 31.8.2014 2.0 as from 1.9.2014	30.0 as from 1.9.2012 until 31.8.2014 12.0 as from 1.9.2014
6.1.6	Smoked sprats and canned smoked sprats[(25)(47)] (*sprattus sprattus*); bivalve molluscs (fresh, chilled or frozen)[(26)]	5.0	30.0
6.1.7	Bivalve molluscs[(36)](smoked)	6.0	35.0
6.1.8	Processed cereal-based foods and baby foods for infants and young children[(3)(29)]	1.0	1.0
6.1.9	Infant formulae and follow-on formulae, including infant milk and follow-on milk[(8)(29)]	1.0	1.0
6.1.10	Dietary foods for special medical purposes[(9)(29)] intended specifically for infants	1.0	1.0

(45) Lower bound concentrations are calculated on the assumption that all the values of the four substances below the limit of quantification are zero.

(46) ***Meat ad meat products that have undergone a heat treatment potentially resulting in formation of PAH, i.e. frying, grilling, barbecuing, roasting and toasting.***

(47) For the canned product the analysis shall be carried out on the whole content of the can. As regards the maximum level for the whole composite product Art. 2(1)(c) and 2(2) shall apply."

Appendix 3 Indigenous chicken breeds in Uganda