KINETIC MODELING AND PARTICULATE CHARACTERIZATION OF SELECTED BIO-HAZARDOUS BY-PRODUCTS FROM HIGH TEMPERATURE PYROLYSIS OF GOAT MEAT

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Award of the of Master of Science Degree in Chemistry of Egerton University

EGERTON UNIVERSITY

MAY, 2016

DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been presented either wholly or in part for the award of a degree in any institution.

Nicholas Rono
SM11/23504/14

Signature…… Date……

RECOMMENDATION

This thesis has been submitted for examination with our approval as supervisors according to Egerton University regulations.

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DEDICATION

It is my honest gratefulness and warmest regards that I dedicate this work to my loving parents Mr. Timothy Toroitich and the late Mrs. Gladys Toroitich, siblings, my wife Faith Rono, and my son Kelvin.

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ABSTRACT

Although molecular toxins and emission of particulates from the thermal degradation of biomass materials such as meat is a complex area of study, it has become necessary to investigate this phenomenon and determine how it affects biological functions and as precursors for cancer, degenerative diseases that stress the respiratory landscape. Accordingly, this study describes the pyrolysis of goat meat in the temperature range 300-525 ˚C. Goat meat sample of 10±0.2 g was heated under atmospheric conditions in an air depleted environment in a thermal degradation reactor (volume ~ 1.6 cm^3) and the smoke effluent passed through a transfer column and collected over 10 mL dichloromethane and kept in crimp top amber vials. Evolution of selected molecular toxins was monitored using an in-line Gas Chromatography hyphenated to a mass spectrometer (GC-MS) while the particulate nature and morphology of particulate emissions was investigated using a scanning electron microscope (SEM). GC-MS analysis was carried out using an Agilent Technologies 7890A GC system coupled to an Agilent Technologies 5975C mass selective detector (MSD). The density functional theory (DFT) and Chemissian softwares were used to explore the thermochemistry and molecular geometries of 2-(ethylthio)phenol, 2,3-dimethylhydroquinone, and 1,1'-biphenyl. The major organic volatiles detected from GC-MS were phenol, 2,3-dimethyl hydroquinone, 2-(ethylthio)phenol, indole and 1,1'-biphenyl. At a pyrolysis temperature of 500 ˚C, the mean particle size of particulates was found to be 7.72 ± 0.61 µm while at 700 ˚C, the particulate size of emissions was found to be 3.52 ± 0.31 µm. This particles are small in sizes and are possible to get into the lungs and cause respiratory problems Moreover, a kinetic model for the thermal destruction of indole, 2,3-dimethylhydroquinone, and 2-(ethylthio)phenol within a temperature region of 450 ˚C and 525 ˚C using pseudo-first order reaction kinetics has been proposed at a residence time of 2.0 s. Consequently, the temperature dependent rate constants for the destruction of 2,3-dimethylhydroquinone, 2-(ethylthio)phenol, and indole were estimated to be, $k = 4.11 x 10^{17} T^n x e^{-\frac{263.10}{RT}}$ s^{-1}, $k = 1.10 x 10^{19} T^n x e^{-\frac{283.38}{RT}}$ s^{-1}, and $k = 1.30 x 10^{21} T^n x e^{-\frac{315.76}{RT}}$ s^{-1} respectively. The electron density maps as well as the toxicity index of 2-(ethylthio)phenol are also reported in this study. The fundamental finding of this study is that most bio-hazardous by-products were evolved in high yields above 300 ˚C.

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ANOVA	Analysis of variance
ATSDR	Agency for toxic substance and disease registry
CP-PAH	Cyclopentafused- polycyclic aromatic hydrocarbon
DFT	Density functional theory
DHHS	Department of health and human services
DNA	Deoxyribonucleic acid
EC	European commission
EC_{50}	Effective concentration where 50% of its maximum effect is observed
EI	Electron ionization
EPA	Environmental protection agency
GC-MS	Gas chromatography mass spectrometer
HACA	Hydrogen addition acetylene abstraction
IARC	International agency for research on cancer
LC_{50}	Lethal concentration required to kill 50% of the population.
NIST	National institute of standard technology
NPAH	Nitro- polycyclic aromatic hydrocarbons
OSHA	Occupation safety and health administration
PAH	Polycyclic aromatic hydrocarbons
PM	Particulate matter
PM_{10}	Particulate matter ≤ 10 μm
$PM_{2.5}$	Particulate matter ≤ 2.5 μm
SEM	Scanning electron microscope
TEM	Tunneling electron microscopy
WHO	World health organization

CHAPTER ONE

INTRODUCTION

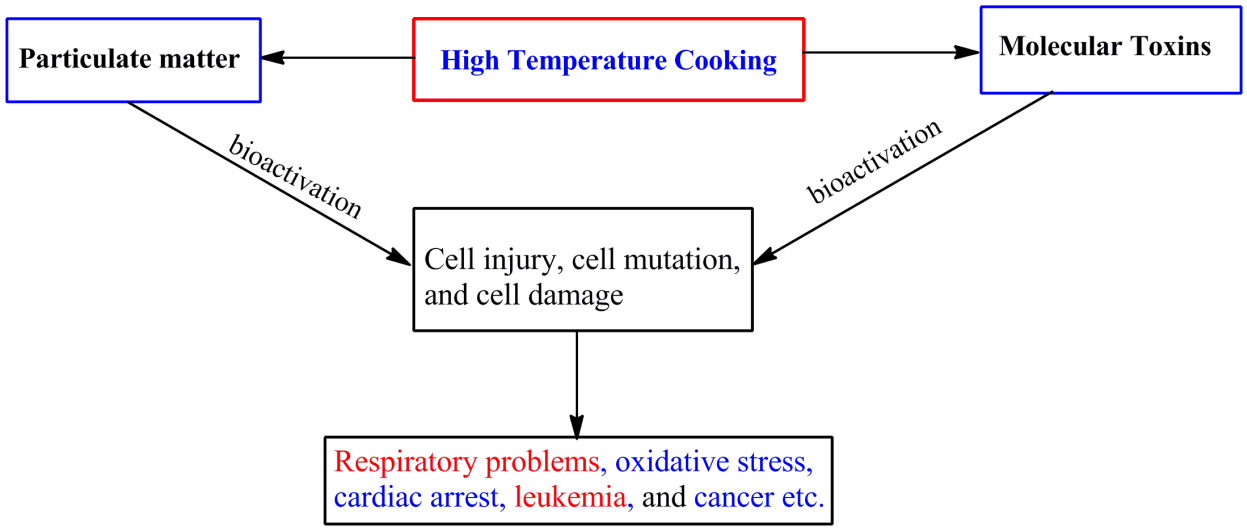
1.1 Background information

Food pyrolysis plays a significant role in several cooking processes such as heating, baking, frying, and grilling. Limited studies have been carried out on the combustion and evolution of molecular toxins from high temperature cooking of red meat. Evidently, during high-temperature cooking of proteinaceous foods such as meat, some bio-hazardous by-products including heterocyclic aromatic amines, phenols, and polycyclic aromatic hydrocarbons (PAHs) are formed (Paula *et al.*, 2004). Therefore, health problems caused by mutagenic and carcinogenic compounds resulting from high temperature cooking and other pyrolysis sources have received mounting global attention. For instance, more than two decades ago, Japanese scientists discovered a new group of highly mutagenic compounds classified as heterocyclic amines (HCAs) from broiled and grilled meat, and fish dishes (Jagerstad *et al.*, 1998). These toxins are formed when meat, including beef, pork, fish, chicken is cooked using high-temperature procedures which may include pan frying or grilling directly over an open flame (Chiang and Quek, 2015). Previous studies have shown that rodents fed on meals spiked with heterocyclic amines (HCAs) developed tumors of the breast, colon, liver, skin, lung, and prostate cancers (Jagerstad *et al.*, 1998).

In the search for possible correlation between diet and cancer, the highly mutagenic heterocyclic amines, phenols, and other organic toxics present in cooked foods have attracted a great deal of attention (Doll and Peto, 1981; Jagerstad *et al.*, 1998). Basically, HCAs are formed when amino acids (the building blocks of proteins), sugars, and creatine (a substance found in muscle) react at high-temperatures (Cross and Sinha, 2004). Heterocyclic amines are capable of damaging DNA when they are metabolized by specific enzymes in the body, following a process called bio-activation which in turn initiates the development of cancer risks and other cellular damaging consequences (Sugimura *et al*., 2004).

The by-products of high-temperature cooking of meat are responsible for serious health problems such as cancer, heart attack, and respiratory problems including emphysema (Bosire, 2015). Phenol derivatives such a *o*-cresol, polycyclic aromatic hydrocarbons including

1,1'-bipenyl and 2-ethenylnaphthalene and heterocyclic amines such as 2,3-benzopyrrole are the subject of investigation in this work. These compounds are suspected to account for the largest burden of illnesses and deaths in developing countries from biological complications (Gibis, 2016). These compounds may be categorized under the family of highly mutagenic compounds. The search for optimum cooking temperatures in order to minimize grave biological impacts, affecting human health and environmental ecosystems is therefore necessary. Scheme 1.1 gives a summary of the generation of particulates and associated bio-hazardous by-products from high temperature cooking and their potential health consequences (See *et al.*, 2006).



Scheme 1.1: A summary of the biological impacts of combustion by-products from high temperature cooking of red (goat) meat

In addition to organic toxins emitted during high temperature cooking of meat, particulate emissions and other airborne pollutants may cause severe health problems which may include injury to respiratory landscape, and lung related problems. Particulate matter (PM) which is a mixture of solid particles and liquid droplets exists in the ambient air either in form of dust, soot, or smoke (Dergham *et al.*, 2015). Biomass burning such as meat grilling results in the emission of particulate matter in form of soot and aerosols (Klasen *et al.*, 2015). These particles come in many sizes and shapes and can be made up of hundreds of different compounds. There are two main categories of particulate matter; PM_{10} ($\leq$ 10 µm) in diameter, and $PM_{2.5}$ which is 2.5µm or less in diameter (Yorifuji *et al.*, 2015). The size of these particles is directly connected with

possible environmental and health risks (Bai *et al.*, 2015). Small particles less than 2.5 micrometers in diameter pose serious problems because they can penetrate deep into the lungs and some may even get into bloodstream hence causing severe damage to biological systems including cell aberrations (Chung *et al.*, 2015).

Although many studies have been conducted on the thermal degradation of various biomass materials, few studies have investigated on the combustion of red meat at high cooking temperatures. In general, when biomass (meat) is subjected to a temperature of about 450 degrees Celsius more toxic volatile compounds are formed. Typically, at about 500 to 700 degrees Celsius, pyrosynthetic formation of PAHs takes place as a result of aromatization, condensation and carbonization of organic material which constitute particulate matter (Lehmann and Joseph, 2015).

Most essential foodstuffs such as meat, fish, milk, oils and fats, are subjected to heat treatment in their preparation resulting in the formation a myriad of toxic compounds (Janzowski *et al.*, 1978). In essence, HCAs, PAHs and phenolic compounds have been classified as carcinogenic materials by many organizations, including the International Agency for Research on Cancer (IARC), the Department of Health and Human Services (DHHS), the National Occupation Safety and Health Administration (OSHA), and the United States Environmental Protection Agency (US-EPA), and ATSDR (Lee and Vu, 2010).

In summary, the following graphical representation (Figure 1.1) illustrates the major chemistry investigated in this study. The graphic gives, techniques, and some of the results reported in this work.

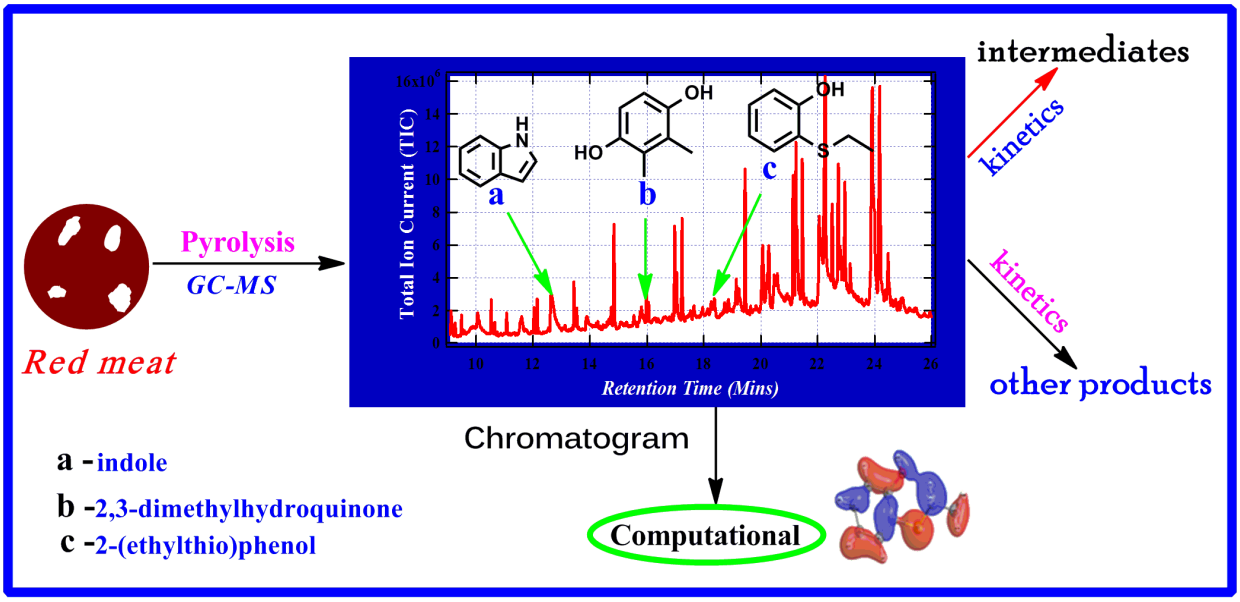


Figure 1.1: Graphical representation of the chemistry presented in this study

1.2 Statement of the problem

Heterocyclic amines, PAHs and phenols are well established carcinogenic, mutagenic, as well as hematoxic classes of compounds. Studies on experimental mice have shown that, PAHs and other classes of organic toxic compounds such phenols and nitro-PAHs cause liver and blood problems. Notably, benzo[a]pyrene in short-term exposure causes birth defects. HCAs, PAHs and phenols are formed during high-temperature cooking of meats, thus exposure to humans is highly possible. Many people around the world are in danger of exposure to toxins for eating well-done meats, fried, and barbecued foods. Grilling and charring of food enhances the formation of HCAs, PAHs, and phenols in muscles. In Kenya for instance, a study was conducted in Kajiado County and showed that most lifestyle diseases including cancer are directly linked to the habit of eating processed, fried or well-done red meat. This study focused on the characterization of volatile components (HCAs, PAHs, phenols) formed over a wide range of cooking temperatures of goat meat as well as characterize particulate emissions released during high temperature pyrolysis of goat meat. This has not been reported in previous studies. Moreover, kinetic modeling of toxins formed from high temperature cooking of goat meat has not been accorded serious attention before. Kinetic modeling gives insight into the possible mechanistic pathways and transformations of toxins into other by-products including free radical formation.

Many Kenyans enjoy eating roasted and grilled red meats unaware of the potential health risks associated with bio-hazardous products formed during high temperature cooking procedures. Thus, there is need to examine experimentally the formations of potentially harmful by-products of goat meat pyrolysis and characterize the particulates.

1.3 Objectives

1.3.1 General objective

To investigate quantitatively the presence of HCAs, PAHs, phenols, characterize particulate matter emissions and develop kinetic model for formation and destruction of selected toxins from high temperature pyrolysis of goat meat.

1.3.2 Specific objectives

1. To Identify and quantitate HCAs, PAHs and phenols, formed at various pyrolysis temperatures of goat meat.
2. To develop a kinetic model for the destruction and formation of selected toxic compounds from high temperature pyrolysis of goat meat.
3. To determine the size of particulate matter from high temperature pyrolysis of goat meat at the temperatures of 500 $^{\circ}$C and 700 $^{\circ}$C using Scanning Electron Microscopy (SEM).

1.4 Hypotheses

1. HCAs, PAHs and Phenols will not be formed in significant amounts during the pyrolysis of goat meat.
2. A kinetic model for the destruction and formation of molecular toxins from the thermal degradation of a heterogeneous system such as meat is not possible.
3. The size of particulate matter from pyrolysis of goat meat at 500 $^{\circ}$C and 700 $^{\circ}$C will not be significantly different.

1.5 Justification

Whereas the mechanistic formation of HCAs, PAHs and phenols in combustion systems is well studied, limited studies have focused on the evolution of these bio-hazardous compounds from high temperature cooking of goat meat over a wide range of cooking temperatures. Furthermore, the focus on the kinetic behaviour of toxic compounds from high temperature cooking has not been addressed in literature. This study therefore is important in developing a kinetic model for

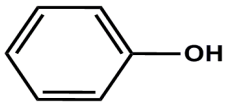
the selected toxin formation characteristic of high temperature cooking procedures. In the search for possible correlation between diet and cancer, the highly mutagenic HCAs, PAHs and phenols present in cooked foods have attracted a great deal of attention. Accordingly, the present studies are fascinated with their formation, occurrence in food products, bio-transformation, and carcinogenicity. The bio-hazardous by-products of high temperature cooking are believed to be responsible for serious health problems such as cancer, heart attack, and respiratory problems including emphysema. The study generated data on the presence of HCAs, PAHs and phenols, and particulate matter characterization from pyrolysis of goat meat at various temperatures.

CHAPTER TWO

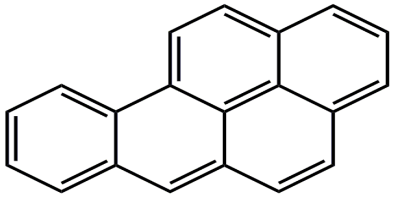
LITERATURE REVIEW

2.1 Meat as a source of protein

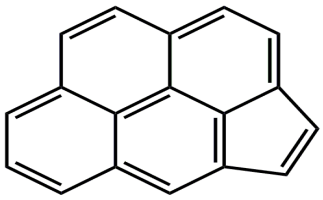
Most communities around the world hold meat with high esteem because of its associated nutritional value (Bosire, 2015). Meat is one of the sources of proteins, among other animal products such as fish, pork, and chicken and primarily contains essential amino acids needed for health growth (Jiménez-Colmenero *et al.*, 2001). Essential amino acids that cannot be synthesized by the human body need to be supplied by protein rich foods including meat (van Vliet *et al.*, 2015). Proteins are very important in the human body, because they are indispensable in body building, repair of body tissues, enzyme production, generation of hormones, and many biological molecules. Furthermore, proteins are also reported as a source of energy (Smil, 2002). Examples of the most notorious class of compounds believed to cause serious health complications are presented in Figure 2.1.



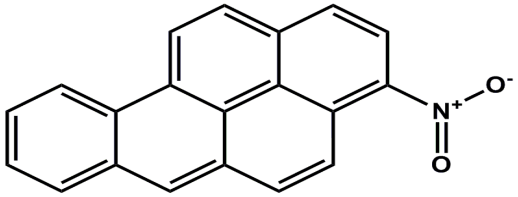
Phenol



benzo[a]pyrene



cyclopenta[c,d]pyrene



3-nitro benzo[a]pyrene

Figure 2.1: Some of the important classes of molecular toxins from combustion of organic matter

Nonetheless, high temperature cooking of meat has been suspected to release biologically harmful components. The evolution of bio-hazardous components of meat during high temperature cooking procedures is the subject of this investigation.

2.2 The Sources of heterocyclic amines, polycyclic aromatic hydrocarbons, and phenols

Heterocyclic amines, PAHs and phenols originate mainly from anthropogenic processes, particularly from incomplete combustion of organic fuels and pyrolysis of biomass materials (Slawomir *et al.*, 2008; Lee and Vu, 2010). Natural processes, such as volcanic eruptions and forest fires, also contribute to ambient existence of PAHs. Polycyclic aromatic hydrocarbons and HCAs can exist as solid particulates or gaseous components depending on their volatility (Lee and Vu, 2010). Certain bacteria can also synthesize PAH derivatives including perylene and benzo[*a*]pyrene (Neff, 1979; Magi *et al.*, 2002), while other forms of bacteria biodegrade PAHs in the soil hence reducing their contamination (Clemente *et al.*, 2001). Combustion of fossil fuels also results in the formation of PAHs and HCAs. Harbours have been known to contain high concentration of petrolytic PAHs probably due to leakage of petroleum based products which may contain PAHs as well as other contaminants (Soclo *et al.*, 2000). Some PAHs such as phenanthrene and anthracene have been found in meteorites (Wing and Bada, 1991). On the other hand, phenols in the natural ecosystems are produced primarily from the degradation of numerous biomass materials, industrial, and municipal waste. Some phenols however, are also produced during accidental burning of oil fields, forest fires, burning of tyres, and volcanic activities (Michalowicz and Duda, 2007). These toxins enter the environment during incomplete combustion or pyrolysis of organic matter, during industrial processes and other human activities for instance grilling of meat (Kuo *et al.*, 2005). Polycyclic aromatic hydrocarbons are also formed in processes, such as carbonization (Beck *et al.*, 1994; Reilly *et al.*, 2000). Human activities such as processing of coal, crude oil, petroleum, and natural gas emit PAHs to the environment (Food safety Authority of Ireland, 2009). Direct contact with contaminated soils (especially in coal mines and industrial sites) leads to an exposure to carcinogenic PAHs (Zhao *et al.*, 2014). Phenol and its derivatives penetrate the environment from sources such as, use of petrol, effluents from pharmaceutical industries, use of pesticides and drainage off of municipal sewage to the surface water (Michałowicz and Duda, 2007).

Heterocyclic amines are formed from the reaction between amino acids, sugars, and creatine at high temperatures. Consequently, large amounts of HCAs are found mainly in muscle meat cooked at high-temperature and in low concentrations in other charred food types (Vangnai *et al.*, 2014). Indole (2,3-Benzopyrrole) is a typical example of heterocyclic amine. Indole is a

suspected carcinogen and produces NOx when heated and is also a well-known eye irritant (Lewis, 2008).

2.3 Human dietary exposure to HCAs, Nitro-PAHs and phenols

The most important dietary source of organic toxins such as PAHs, phenols, and HCAs is the high consumption of meat cooked over an open flame (Phillips, 1999). Pyrolysis of fat drenched from food onto hot charcoal, or a related heat source, can also be a significant source of PAHs, HCAs, and phenols in grilled or barbecued foods (Fernandes *et al.*, 2015). Studies conducted in Catalonia, Spain, showed that PAH ingestion from cooked meat and other dietary supplements was far above the recommended levels. For instance the concentration of PAHs in male adults, adolescents, children, seniors, and female adults were respectively, 8.4 µg/day, 8.2 µg/day, 7.4 µg/day, 6.3 µg/day, and, 6.3 µg/day (Falco *et al.*, 2003; Martorell *et al.*, 2010). Therefore, processing of meat and dairy products, such as charcoal grilling, roasting and smoking are the major sources of PAHs in protein related foods (Wenzl *et al.*, 2006). The major routes of exposure to PAHs, HCAs, and phenols are from food and inhaled air (Food safety Authority of Ireland, 2009; Lee and Vu, 2010).

2.4 Pyrolysis process of organic materials and associated by-products

Pyrolysis is the form of treatment that chemically decomposes organic materials by heat in the absence of oxygen (Puy *et al.*, 2011). Pyrolysis of materials proceeds through a series of complex reactions and yields a variety of products (Shafizadeh, 1982; Bridgwater *et al.*, 1999). Bio-oils for instance, when pyrolized yield PAHs such as naphthalene, acenaphthylene, acenaphthene, fluorene and pyrene (Tsai *et al.*, 2009). Pyrolysis products can give an insight of the component or treatment of the residues, for example presence of alkane/alkene blueprint in several residues and in the ceramic material of the potsherd is interpreted as the pyrolysis result of an aliphatic arrangement that has been produced from foodstuffs under high temperature cooking procedures (Oudemans, 1991). Temperature and the substrate composition are the primary pyrolysis conditions required for the formation of organic by-products of combustion. Increasing the temperature causes an increase in the amount of PAHs, HCAs and/or phenols formed (Sharma and Hajaligol, 2003). Pyrolysis experiments can be conducted in isothermal laminar-flow quartz-tube reactors or in shock-tubes (Poddar *et al.*, 2011).

2.5 Nitro-polycyclic aromatic hydrocarbons (Nitro-PAHs)

Another category of combustion by-products of biomass materials including meat is the notorious nitro-polycyclic aromatic hydrocarbons (nitro-PAHs). They comprise one of the most troubling classes of environmental pollutants, found in air, aquatic systems, grilled food, and sediments (Reichardt *et al.*, 2009). Nitro-PAHs are released to the environment as a result of direct emissions from incomplete combustion processes and high temperature cooking, but are also formed in situ in the atmosphere by gas phase oxidation and nitrite ($NO_3^{\bullet}$) radical reactions of PAHs (Monge *et al.*, 2010). Consequently, Nitro-PAHs are well-known as mutagenic and carcinogenic compounds, and they pose serious health risks because of their persistence in the environment (Cohen, 2000). The most efficient way of removing Nitro-PAH from the environment is natural photochemical degradation process but this may result in the formation of oxidation products which are more harmful than the original nitro-PAH. More importantly, the study of the excited state relaxation pathways is an indispensable initial step towards understanding the photochemical fate of nitro-PAHs in the environment (Reichardt *et al.*, 2009).

2.6 The toxicological implications of HCAs, phenols, PAHs, and nitro-PAHs

2.6.1 The toxicity of HCAs

As earlier described, heterocyclic amines (HCAs) are chemicals formed when muscle meat, including beef, pork, fish, or poultry, is cooked using high-temperature methods, such as pan frying or grilling directly over an open flame (Chiang and Quek, 2015). In laboratory experiments, HCAs have been found to be potent mutagenic and carcinogenic compounds (Budhathoki *et al.*, 2015).

2.6.2 The toxicity of nitro-PAHs

Nitro-PAH is more mutagenic than oxy-PAHs and other PAHs as reported in studies conducted in the urban area of the São Paulo city, Brazil, on salmonella assay (Umbuzeiro *et al.*, 2008). 1-nitronaphthalene (Nitro-PAH) has been reported to have caused acute toxicity to aquatic organisms, with an LC_{50} (96 h) of 9.0 mg/L reported for the sheepshead minnow (*Pimephales promela)* (Onduka *et al.*, 2012). Besides, this nitro-PAH inhibited the growth of the ciliate *Tetrahymena pyriformis*, with an EC_{50} (60 h) of 17.3 mg/Litre (WHO, 2003; Shen, 2014).

Nitro-PAHs have also been reported to be carcinogenic and mutagenic for instance, 3-nitro-benzo[a]pyrenes are potent mutagens in Salmonella (Fu *et al.*, 1994). In some cases, they stimulate birth defects (Sims *et al.*, 1988). These compounds can be transported in the vapour phase or adsorbed onto particulate matter hence increasing chances of exposure to both animals and human beings (Ma *et al.*, 2015). Furthermore, nitro-PAHs have been detected in the emissions of kerosene heaters, fuel gas and petroleum burners used for heating and cooking at home, as well as in the fumes of cooking oils, and this has therefore lead to indoor exposure of nitro-PAHs in poorly ventilated conditions (Shen, 2014).

Studies done in Athens, showed that nitro- PAHs were fixed in soot and this was mainly, 2-nitrofluoranthene and 2-nitropyrene which are well known as mutagenic and carcinogenic (Marino *et al.*, 2000). High Meat intake, and more so the red meat, has been indeed linked with pancreatic cancer (Stolzenberg-Solomon *et al.*, 2007) and has been attributed to high-temperature cooking of meat, which in turn results in formation mutagens and other toxins (Zheng and Lee, 2009). Typical examples of these compounds includes 3-nitro-benzo[a]pyrene and 2-(3,4-dimethoxyphenyl)ethylamine

2.6.3 The toxicity of phenols

Phenol is a colourless crystalline substance with a characteristic smell, and is soluble in water and organic solvents (Sadasiva, 2015). Because of its high toxicological implications on humans and natural ecosystems, it was among the first pollutants to be included in the list of priority pollutants by the US-Environmental Protection agency (US-EPA) (Chand Meena *et al.*, 2015). Vehicular exhaust as well as the use of phenol-based pesticides release phenol and its derivatives to the environment (Pan and Miao, 2015). Nevertheless, phenol and its derivatives may be present in foods. Interestingly, a small amount of this substance was found in honey and also in coffee (Michałowicz and Duda, 2007). The concentration of phenols in processed food may reach an alarming concentration level. For instance, in grilled pork and sausage, phenol content was reported to be 28.6µg/kg and 7µg/kg respectively (US EPA, 1980). Phenols have been found on the charred surface of smoked meat (Trafialek and Kolanowski, 2014). Moreover, Phenols are formed in the liver when ingested benzene is metabolized to phenolic ring-opened products and its derivatives (Snyder *et al.*, 1993). The fact that the molecular phenolic compounds from

combustion sources such as high-temperature cooking are a threat to environmental ecosystems and health is a subject of global concern.

The toxicity of phenolic compounds is associated with two main processes – unspecified toxicity related to hydrophobicity of the individual compound and formation of free radicals (Michałowicz and Duda, 2007; Dellinger *et al.*, 2007; Kibet *et al.*, 2015). Hydrophobicity affects the solubility of phenol in a cell and, thus, the possibility of interaction of the compound with specified cell and tissue structures (Michałowicz and Duda, 2007). Hydrophobic compounds can cross biological barriers which contain lipids, for example, cell or microsomal membranes and skin stratum corneum (Debnath *et al.*, 1994; Smith *et al.*, 2000).

2.6.4 Toxicity of PAHs

Polycyclic aromatic hydrocarbons (PAHs) are included in priority pollutant lists because of their mutagenic and carcinogenic properties (Kuo *et al.*, 2005; Muyela, 2012). Numerous studies have shown that exposure to benzo[a]pyrene, for instance, increases the risk of cancer (Muyela, 2012). PAHs are mutagenic and also teratogenic (Lee and Vu, 2010).

Previous studies have shown that treatment of whole animals with benzene leads to decrease in the levels of circulating blood cells (Snyder *et al.*, 1993). Lymphocytes are a target of benzene, with their depletion occurring early in the course of benzene exposure (Ward *et al.*, 1996; WHO 2010). The readily detected cytogenetic abnormalities in lymphocytes following significant benzene exposure clearly indicate that lymphocyte DNA is affected as a result of benzene exposure (Eastmond *et al.*, 1994). Benzene and phenol are also known to produce chromosome damage as well as cell injury (Pollini and Colombi, 1964). Benzo[a]pyrene in particular has shown various toxicological impacts such as haematological effects, reproductive and developmental toxicity and immunotoxicity on experimental animals (Food safety Authority of Ireland, 2009). During meat grilling, PAHs are produced as soon as fat and juices from meat grilled directly above an open fire drip onto the fire, causing flames. These flames contain PAHs that then stick onto the surface of the meat (Cross and Sinha, 2004). Smoked meat that is widely consumed by people all over the world contain substantial amounts of PAH and derivatives (Chen *et al.*, 2014). In a study conducted on Malaysian grilled meat dishes fluoranthene was found in all samples (Afsaneh *et al.*, 2010).

PAHs have been reported to be carcinogenic (Yang *et al.*, 2014), mutagenic (Mara Fisner *et al.*, 2013) and also genotoxic to humans (Johnsen *et al.*, 1997). Since PAHs are lipid soluble, they distribute in the body to various organs through the liver, lungs and subcutaneous fat layer. Toxicity equivalent factors have been used to estimate the toxic ability of individual PAHs with reference to Benzo[a]pyrene (Yoon *et al.*, 2007). Studies conducted by IARC in the 1980s on PAH work-related exposures showed that an explicit risk of cancer was found in workers employed in coke (lung cancer), aluminum (lung and bladder cancer), and steel industries (lung cancer) (Mastrangelo *et al.*, 1996; Boffetta *et al.*, 1997).

Moreover, PAH mutagenicity is accelerated by activating the bay region of a PAH (Pahlman and Pelkonen, 1987). Inorganic ions, co-enzymes, amino acids, and saccharides may well co-exist with toxic bio-active PAHs in the cell hence raising the toxicity of PAH on DNA (Wang and Yu, 2005). Benzo[a]pyrene binds to DNA and causes gene mutations, and chromosome aberrations in cultured human cells (Peltonen and Dipple 1995). Acenapthylene is a toxic pollutant and its concentration in water is highly monitored (Pitt *et al.*, 1995).

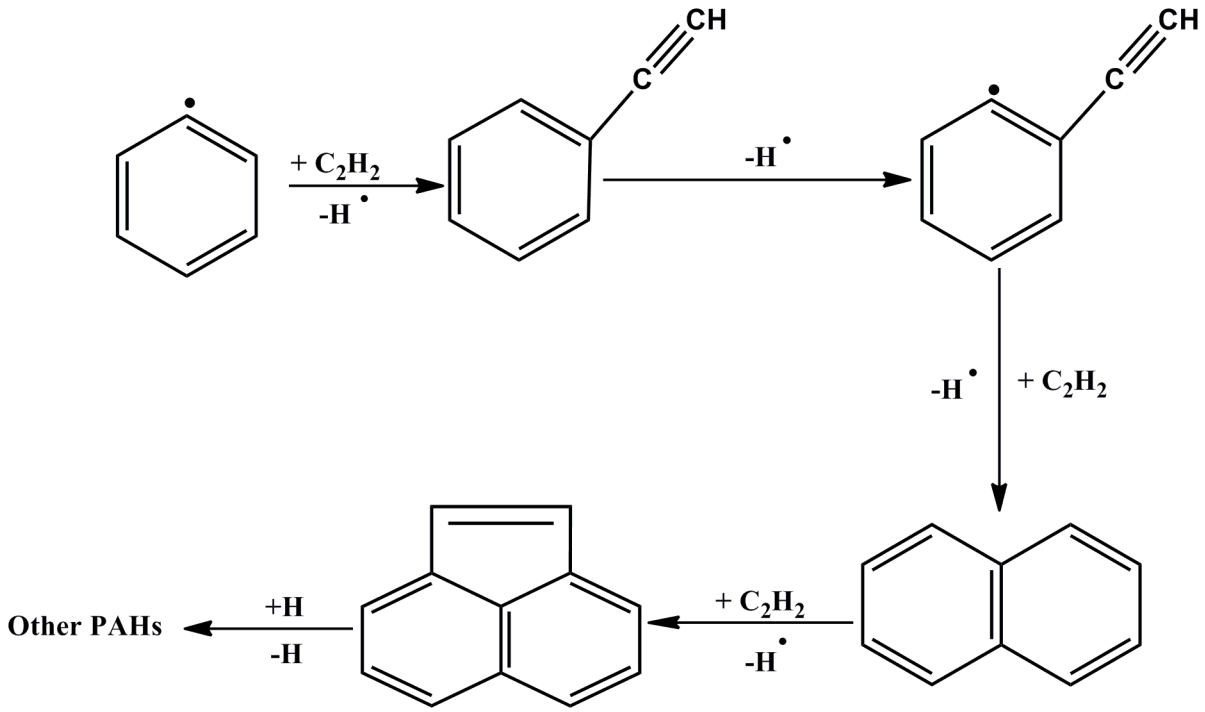
2.7 Cyclopentafused polycyclic aromatic hydrocarbons

Cyclopentafused polycyclic aromatic hydrocarbons are a particular sub-class of PAHs, which contain at least one superficially unsaturated five-membered ring annulated to a PAH perimeter (Mar´ıa and Vlietstraa, 2005). They are ever-present environmental pollutants and prospective human health biohazards (Wang *et al.*, 1999). Cyclopenta[c,d]pyrene is typically a pyrolysis by-product (Lafleur *et al.*, 1993) and is the most studied because of its high toxicological consequences (Goldt, 1978). It is highly carcinogenic and has tumour initiating potential (Mar´ıa Jos´e Otero-Lobatoa and Vlietstraa, 2005). Cyclopentafused PAHs are more bio-active than their analogous PAHs without the cyclopenta moiety (Mar´ıa Jos´e Otero-Lobatoa and Vlietstraa, 2005). For instance, cyclopenta[c,d]pyrene is more bio-active than pyrene. Cyclopentafused PAHs have shown mutagenicity and carcinogenicity in *Salmonella typhimurium* and in Chinese hamster V79 cells with metabolism taking place in the cyclopenta ring (Nesnow *et al.*, 1984; Nyholm *et al.*, 1996). Most identified biologically active CP-PAH requires exogenous metabolic bio-activation to exert positive mutagenic activity in bacterial mutagenicity assays (Ball *et al.*, 1989). Cyclopentafused PAHs are also known to be genotoxic and are believed to interfere with

the genetic make-up of an organism (Brzuzan *et al.*, 2013). A study conducted on rat cell showed increased level of DNA adduct in clara cells and macrophages (Johnsen *et al.*, 1997).

2.8 The mechanistic pathways for PAH formation

Scientists have proposed that PAHs are formed through hydrogen addition acetylene abstraction (HACA) mechanism, as shown in scheme 2.1 (Saha *et al.*, 2010; Cherchneff 2010).



Scheme 2.1: Mechanism of acenaphtylene (PAH) formation by hydrogen abstraction acetylene addition (HACA) process (Cherchneff, 2010).

The HACA mechanism has been shown to generate mostly cyclopentafused PAHs instead of PAHs with six-membered rings only (Kislov *et al.*, 2013). At high Temperatures organic components are partially broken down to smaller but relatively unstable radicals which then recombine to form lager and many stable PAHs (Kabizinski and Cyran, 2002).

2.9 Surface morphology and size of emissions from high temperature cooking of meat

The particulate size and morphology of airborne pollutants is explored using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Transmission electron microscope produces images via the transmission of a focused beam of electrons through a

sample, and forms an image in a comparable way to a light microscope. Transmission electron microscope imaging has considerably higher resolution because electrons are used to illuminate the sample (Horisberger, 1981; Williams and Carter, 1996).

On the other hand scanning electron microscopy scans the sample surface by use of a very fine metallic tip. The tip is mechanically linked to the scanner. The sample may be positively or negatively biased so that a small tunneling current flows if the tip is in contact with the sample. The weak tunneling current is amplified and measured. The tip is scanned by a line above the sample surface following the topography of the sample (Horisberger, 1981; Williams and Carter, 1996).

2.10 Particulate matter and their health implications

Particulate matter is the general term used for liquid droplets, aerosols, soot, smoke, fumes ash and solids mixtures (Su, 1996). They are classified according to size mainly because of the different health effects associated with different diameters. Particulate matter that is 2.5 micrometers in diameter and less is classified as $PM_{2.5}$ while those with 10 micrometers and less are classified as PM_{10} (Tecer *et al.*, 2008). $PM_{2.5}$ is more toxic than PM_{10} because they penetrate deep into respiratory system tissues hence causing lung diseases, and asthma. Nevertheless, PM_{10} can reside on the lungs and result in complications and damage to alveoli (Terzano *et al.*, 2010).

While the health consequences of organic by-products of combustion are well documented in literature, the toxicology action of soot particulates is yet to be understood (Mehmet and Hasan, 2009). Stabilized structures of the reacting radicals of hydrocarbons with conjugated structures and their derivatives are critical intermediates to soot nucleation (Frenklach *et al.*, 1983). For instance, in the pyrolysis of benzene, the aromatic growth is initiated by the formation of biphenyl (Perez, 1991).

2.11 Computational chemistry

Molecular modeling comprises all theoretical methods and computational techniques used to model or mimic the behaviour of molecules (Badrinarayan *et al.*, 2015). The methods are used in the fields of computational chemistry, drug design, computational biology and materials science for studying molecular systems ranging from small chemical systems to large biological molecules and material assemblies (Ochtorski, 2000). The simplest calculations can be

performed by hand, but inevitably computers are required to perform molecular modeling of any reasonably sized system (Vishveshwara, 2014). The common characteristic of molecular modeling techniques is the atomistic level description of the molecular systems. This may include treating atoms as the smallest individual unit (the Molecular mechanics approach), or explicitly modeling electrons of each atom (the quantum chemistry approach) (Jensen, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Reagents and chemicals

All chemicals and reagents used in this study were of analytical grade unless otherwise stated.

3.2 Equipment, instruments, and computational softwares

Agilent Technologies 7890A GC system connected to an Agilent Technologies 5975C MS system, A JEOL (JMS 7100F) scanning electron microscope (SEM), Reactor system, stopwatch and electronic beam balance were used in this work. Gaussian '09, HyperChem, and Chemissian computational softwares were used for geometry optimization of molecules, thermochemistry, and electron density contour maps of frontier orbitals of molecular toxins.

3.3 Sample collection

A fresh sample of goat meat was purchased from a slaughter house near Egerton University and was used without further treatment. All samples were analyzed in replicates of two in order to enhance reliability and reproducibility of data.

3.4 Sample preparation

Goat meat sample from the hind leg (steak) was cut into small pieces of weight 10±0.2 g. Since meat pyrolysis is heterogeneous, it was necessary to conduct replicate samples for every cooking temperature under conditions which simulate actual cooking temperatures. Particulate emissions were trapped using gas trapping apparatus, and stored in amber vials for analysis using scanning electron microscopy. The gaseous products were collected and dissolved in dichloromethane in 50 mL glass beaker. The dichloromethane solution was kept in vials.

3.5 Standards for calibration of molecular products

Standards of purity ≥ 99% were purchased from Sigma Aldrich Inc., UK. Stock solutions in the range of 10μg/L-100μg/L of 2-ethenylnaphthalene,1,1'-biphenyl, indole, 2,3-dimethylhydroquinone, phenol, 2-(ethylthio)phenol were prepared by dilution of respective stock solutions, wrapped with aluminum foil to avoid possible degradation by light, and stored in 10 mL volumetric flasks in a freezer whose temperature was set at 4 ˚C.

3.6 The combustion reactor system

The reactor used was a muffle furnace (Thermo-Scientific), USA whose internal heating compartment has dimensions of 5 cm x 6 cm x 8 cm. The heater is fitted with a temperature regulating knob. The temperature of the heater ranges from 20 ˚C to 1000 ˚C. At the top of the heating compartment is a hole which allows the gas-phase trapping tube to pass through. The sample holder in the heating compartment was quartz reactor of dimensions: i.d. 1 cm x 2 cm (volume ≈ 1.6 cm^3) fitted with gas collecting tube which can withstand high temperatures of up to 1200 ˚C. The reactor system used in this study is presented in Figure 3.1.

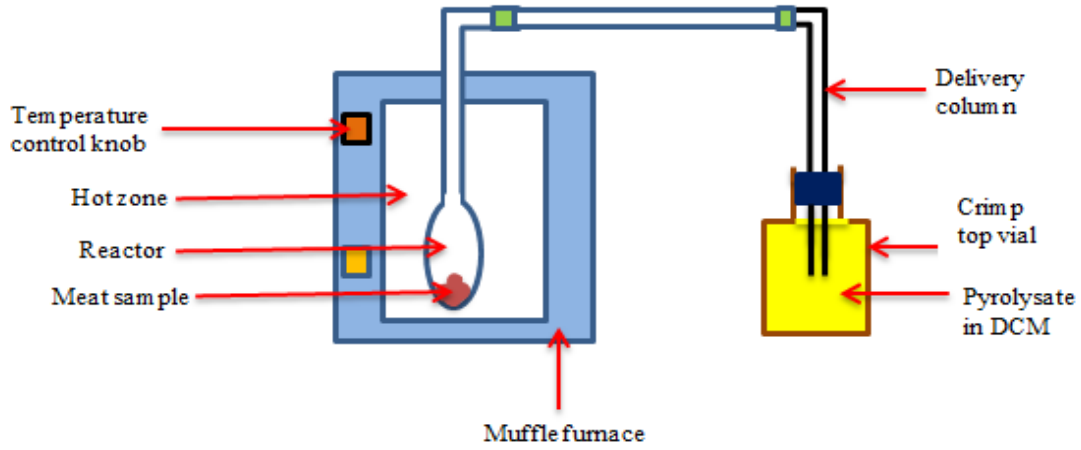


Figure 3.1: The reactor assembly and the gas-phase trapping apparatus

3.7 Pyrolysis of red (goat) meat

Goat meat of mass 10±0.2 g was weighed and carefully packed in a quartz reactor. The meat in the reactor was then pyrolyzed at a constant temperature of 450 ˚C at different residence times of 10, 20, 30, 40, 50, and 60 minutes to monitor the kinetics for the formation of selected molecular toxins. In this study, the formation of 2-(ethylthio)phenol was kinetically investigated as a representative toxin in meat pyrolysis. Gas-phase reaction products were passed through 10 mL dichloromethane solution in 50 mL conical flask. The contents in the conical flask were then transferred to amber vials for GC-MS analysis. The data obtained in this study were averaged from two replicates.

For quantitative determination of PAHs, phenols, and HCAs formed at different temperatures the same sample size of goat meat (10±0.2 g) was heated at various temperatures; 300, 350, 400,

450, 500 and 525 ˚C at a constant residence time of 15 minutes (to simulate the average cooking time in meat joints and homes). The residue formed at every pyrolysis temperature was collected and weighed in order to determine the yields of char. The gaseous products were collected using gas collection glass-tubes connected to the reactor and dissolved directly in 10 mL dichloromethane (DCM). All the samples collected were kept in a freezer at 4 ˚C for analysis using GC-MS.

3.8 Scanning electron microscopy (SEM) analysis

Particulate emission at 500 ˚C and 700 ˚C from meat pyrolysis at a residence time of 15 minutes was dissolved in dichloromethane through a porous tube diluter and transferred into amber vials. About 5 mg of the particulate sample was added to 1 mL methanol and gold grids were dipped into the prepared sample. Twisters were used to pick the gold grids from the sample. The grids were allowed to dry in the open before putting them into the analysis chamber of the SEM (JEOL JMS 7100F), Figure 3.2, *vide infra*.

Figure 3.2: A photograph of a scanning electron microscope (JEOL, JMS 7100F) used in this study (Courtesy of the Department of Mechanical Engineering, University of Surrey, UK)

The sample was analyzed under high vacuum to ensure no interference of air molecules during analysis. The SEM machine was then switched on and imaging of the sample conducted. The lens was varied at various resolutions until a clear focus of the sample was observed. *Image J* computer program was used to determine the size of the soot particles and a distribution curve of particulate size was then determined using *Igor* 5.0 computer software. The mean sizes of the soot particles at 500 and 700 ˚C were reported and presented as Gaussian distributions, where the peak of the curve showed the average of the particle size.

3.9 GC- MS Characterization of molecular products

The quantitative analysis of 2,3-dimethylhydroquinone, 2-(ethylthio)phenol, indole, 2-ethenylnapthalene, and 1,1'-biphenyl was carried out using an Agilent Technologies 7890A GC system connected to an Agilent Technologies 5975C inert XL Electron Ionization/Chemical Ionization (EI/CI) with a triple axis mass selective detector (MSD), The temperature of the injector port was set at 200 ˚C to vaporize the organic components for GC-MS analysis. The carrier gas was ultra-high pure (UHP) helium (99.999%). The column temperature was programmed at 15 ˚C/min to 200 ˚C, held for 1 minute, then at 25 ˚C/min to 300 ˚C and held for 10 minutes. A fused silica capillary column (cross-linked 5% phenyl methyl siloxane; 30 m x 250 µm, df 0.25 µm) (Hewlett-packard) was used. Electron Impact ionization energy of 70 eV was used. To ensure that the right compounds were detected, standards were run through the GC-MS system and the peak shapes and retention times compared with those of 2,3-dimethylhydroquinone, 2-(ethylthio)phenol, indole, 2-ethenylnapthalene, and 1,1'-biphenyl . Standards for selected long chain molecular products were also prepared. The data was run through the NIST library database as an additional tool to confirm the identity of compounds (Kibet *et al.*, 2012). Experimental results were averaged replicates of two.

For accurate and consistent analysis, before any run was made, the mass spectrometer was tuned to check for leaks and water levels in the instrument which would affect the accuracy of the data. This procedure was very important in order to prevent contamination, and extend the life of the EI filament. Quantitative transport tests were initiated before any run was conducted to ensure that there were no leaks in the GC-MS system and guarantee the pyrolysis system is clean. The flow rate of mobile gas in the transfer line was monitored to make sure that it was constant and did not fluctuate. If the flow rate was not consistent, and the pressure was not stable when the

transfer line was connected to the GC-MS then leaks could be present in the system. This was corrected before any experiment could begin. To correct for any leaks in the system, a gas leak detector was used. Whenever leaks were detected along the gas lines, transfer lines, or reactor-injection port interface, the connections were tightened and quantitative transport experiment repeated to make sure no leaks were in the system.

3.10 Computational modeling of selected organic toxins

The thermochemical properties of 2-(ethylthio)phenol, 2,3-dimethylhydroquinone, and 1,1'-biphenyl and were conducted using Gaussian '09 computational platform and Chemissian modeling software. The Density functional Theory (DFT) analytical gradient with B3LYP energy functional and 6-31G basis set was used to compute the energetics of these compounds and their corresponding free radicals. All the computational calculations were conducted at 298.15 K and 1 atmosphere pressure. To compute the energy change for the formation of a compound or a free radical from its constituents, the following thermodynamic equation was applied (Ochtorski, 2000).

$$\Delta rH^0 = \sum\left(\varepsilon_0 - H_{corr}\right)_{products} - \sum\left(\varepsilon_0 - H_{corr}\right)_{reactants} \quad 3.1$$

where, ΔrH^0 is change in enthalpy of the reaction, H_{corr} is the correction to the thermal enthalpy, while ε_0 is the sum of electronic and thermal enthalpies

Geometry optimization calculations were conducted using DFT/B3LYP analytical gradient using the 6-31G basis set. Chemissian computational software was used to develop molecular orbital energy-level diagrams, electronic density contour maps and the determination of the band gap energy for selected molecules.

3.11 Development of the kinetic model for the formation and destruction of molecular toxins

Developing a kinetic model for the destruction kinetics of selected organic toxins, Figure 3.3 was considered based on the following fundamental assumptions: (i) initially, the rate of formation of molecular product prevails the rate of destruction, (ii) at the peak of the curve (maximum concentration of the reaction product) the rates of formation and destruction are approximately

the same, and (iii) after the maximum point, the rate of destruction overwhelms the rate of formation.

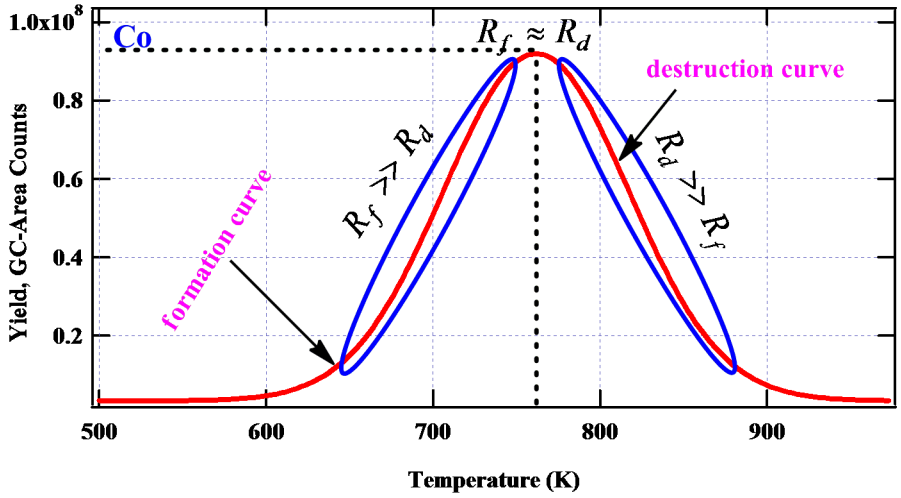


Figure 3.3: The relationship between the rates of formation of the intermediate product (R_f) vs. the rate of destruction (R_d) and Co the maximum concentration of the reaction product.

Based on these assumptions, it is possible to calculate the apparent kinetic parameters for the destruction of pyrolysis reaction products from the temperature dependence of 6its yields. Although, the results obtained in this study are estimated from experimental data and may require further tests, perhaps this is an important step in the study of kinetics from high temperature cooking. In this work, GC-Area counts have been used to determine the destruction rate constants. According to first order reaction kinetics (equation 3.2, *vide infra*) the ratio of concentrations at various temperatures is a constant. Therefore, calibration of the compounds under investigation will still achieve similar results.

For simplicity, a consecutive first order reaction characterized by rate constants k_1 and k_2 is suggested in which a global kinetic model (Taylor *et al.*, 1990; Taylor *et al.*, 1996; Dellinger *et al.*, 2010) is employed to obtain the kinetic parameters. Accordingly, pseudo-unimolecular reactions were applied in which the empirical rate of decomposition of the initial product was first order and expressed by equation 3.2.

$$C = C_o e^{-Kt} \qquad 3.2$$

where: C_o and C are respective concentrations of the reactant at time, t = 0, and time, t = 2.0 s, while k is the pseudo-unimolecular rate constant in the Arrhenius expression (cf. equation 3.2)

$$k = Ae^{-\frac{Ea}{RT}} \qquad 3.3$$

The integrated form of the first order rate law (cf. equation 3.4) was used to calculate the rate constant for the pyrolysis behaviour of toxic compounds from goat meat at a reaction time of 2.0 seconds.

$$k = \ln\left(\frac{Co}{C}\right)\frac{1}{t} \qquad 3.4$$

The activation energy was determined from the Arrhenius plots ($\ln k$ vs. $1/T$) which establishes a linear relationship between the pre-exponential factor A and the rate constant k as given by equation 3.5, where $\ln A$ is the y-intercept and $-\frac{Ea}{RT}$ is the slope.

$$\ln k = \ln A - \frac{Ea}{RT} \qquad 3.5$$

A simple single step reaction mechanism was considered during the thermal degradation of molecular toxins as presented in equation (3.6). Although high temperature pyrolysis of meat is very complex, some understanding on the kinetic behaviour of certain reaction products from basic kinetic equations can be deduced. Therefore, a simple model based on relevant assumptions can be applied.

$$molecular\ toxin \xrightarrow{k_1} I \xrightarrow{k_2} product \qquad 3.6$$

Conventionally, the differential rate laws for each species; *molecular toxins*, intermediate (*I*), and the final *product* are given by equations 3.7, 3.8, and 3.9 respectively.

$$\frac{d[molecular\ toxin]}{dt} = -k_1[molecular\ toxin] \qquad 3.7$$

$$\frac{d[I]}{dt} = k_1[molecular\,toxin] - k_2[I] \quad 3.8$$

$$\frac{d[product]}{dt} = k_2[I] \quad 3.9$$

When these equations are solved analytically, they yield integrated rate laws given by equations 3.10 and 3.11.

$$[tmolecular\,toxin] = [molecular\,toxin]_0 e^{-k_1 t} \quad 3.10$$

Equations 3.11 and 3.12 give the respective concentrations of the intermediate *I* and the *product* at any time t.

$$[I] = \frac{k_1[molecular\,toxin]_0}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \quad 3.11$$

$$[product] = [molecular\,toxin]_0\left[1 + \frac{k_1}{k_1 - k_2}\left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t}\right)\right] \quad 3.12$$

In order to simplify equation 3.11 further, a step two (equation 3.6) is assumed as the rate determining step so that $k_1 > k_2$ and thus the term $e^{-k_1 t}$ decays more rapidly than the term $e^{-k_2 t}$. Therefore equation 3.12 reduces to equation 3.13. This assumption was valid based on previous studies documented in literature (Zhang *et al.*, 2011; Zhang *et al.*, 2012).

$$[product] = [molecular\,toxin]_0\left(1 - e^{-k_2 t}\right) \quad 3.13$$

Moreover, the rate of formation of 2-(ethylthio)phenol from high temperature cooking was explored experimentally at modest cooking times (10, 20, 30, 40, and 50 minutes). For every proposed cooking time, the concentration of 2-(ethylthio)phenol was determined using a GC hyphenated to a mass selective detector (MSD).

In order to calculate the rate constant k_2 for the formation of phenol from the addition of an H radical to the intermediate (2-hydroxybenzyl radical), the differential rate law provided in equation 3.13 was used based on the assumptions that; the major by-product from the destruction of 2-(ethylthio)phenol must be phenol. This assumption is valid if we consider the

reactive nature of the H radical in the radical pool present in combustion systems relative to the methyl radical (Kibet *et al.*, 2012). The other assumption is that *o*-cresol and ethanethiol are minor products. From an experimental perspective, this assumption is true because neither *o*-cresol (2-methylphenol) nor ethanethiol were detected in the entire temperature range of meat pyrolysis but phenol was detected in significant amounts, (Figure 4.1A and Figure 4.10), *vide infra*.

3.12 Statistical analysis of data

A one way analysis of variance (ANOVA) was used to test if there was significant difference in the mean diameters of particulate emissions formed at different temperatures. The Minitab software *ver*. 16 was used to test the difference is significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Molecular distribution of organics at various cooking temperatures of goat meat

Whereas many reaction products are evolved during the thermal degradation of a heterogeneous organic material such as meat, only pyrolysis products that are evolved in significant quantities but are also toxic to both human health and environmental ecosystems were considered in this work. Various classes of organic toxins were released from the combustion of goat meat. Nearly all organic toxins reached a maximum yield at about 450 ˚C as presented in Figure 4.1.

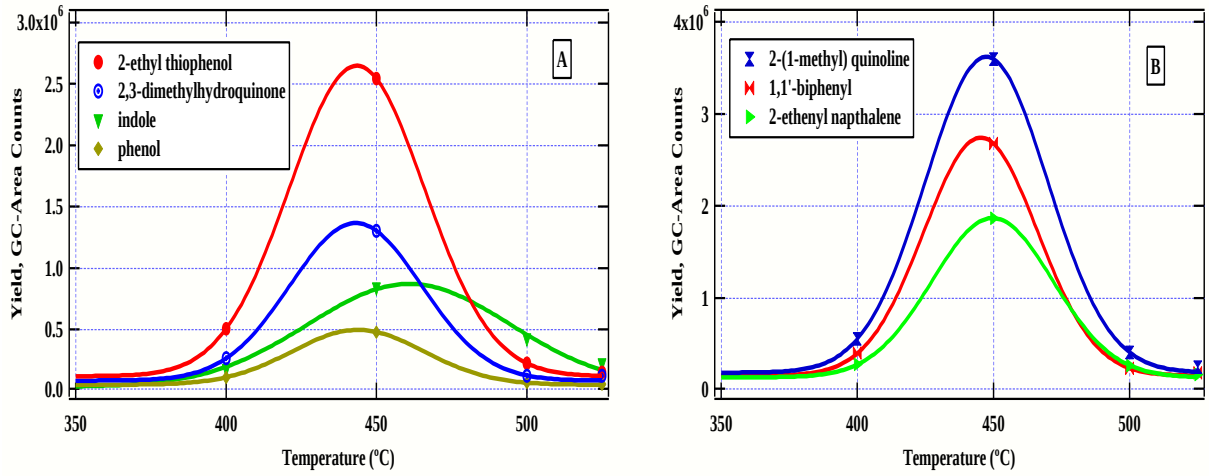


Figure 4.1 Yield (GC – Area counts) distribution of molecular toxins from high temperature cooking of goat meat.

The major toxic group of compounds in the heterocyclic amine category were 2-(1-methyl)quinoline and indole with respective maximum GC –Area counts of 5.60 x 10^6 and 8.39 x 10^5 (Figure 4.1A and B). In the phenolic category, 2-(ethylthio)phenol gave the highest yield at 450 ˚C of 2.54 x 10^6 GC – Area counts, Figure 4.1A. On the other hand, 1,1'-biphenyl and 2-ethenylnapthalene in the class of PAHs were the major products giving GC – Area count yields of 2.68 x 10^6 and 1.88 x 10^6 at 450 ˚C (Figure 4.1B) respectively. Other major products included 2,3-dimethylhydroquinone and phenol. Below 400 ˚C most molecular toxics were released in low quantities. Therefore, these results suggest a safe cooking temperature region of about 300 ˚C or even lower. Although, it appears that at high temperatures > 450 ˚C, the yields

of reaction products decrease, the resultant residue is largely char which is basically aromatic and therefore non edible. The yields of virtually all pyrolysis products investigated in this study, increase sharply at 400 ˚C and decrease suddenly above 450 ˚C (Figure 4.1).

The quantitative release of bio-hazardous compounds was monitored using a GC-MS and presented in Figure 4.2. The structures as well their retention times, and molecular masses are presented in Table 4.1, *vide infra*. Long chain molecular compounds were also detected and presented in Figure 4.3. The overlay chromatograms at 400 ˚C and 500 ˚C show clearly the intensity of each reaction product at various retention times.

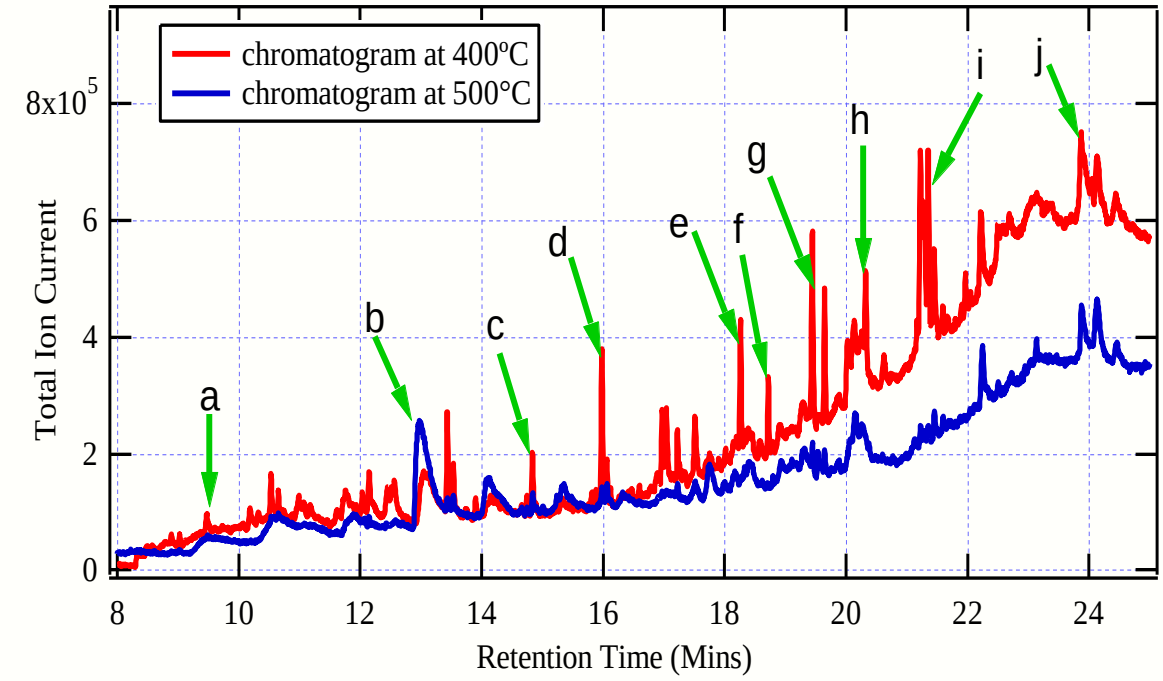
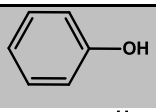
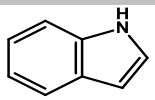
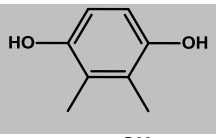
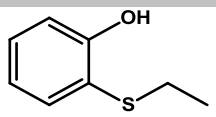
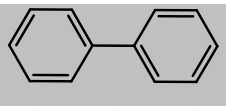
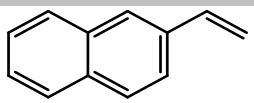
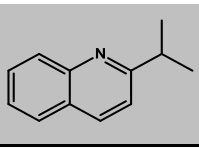


Figure 4.2: GC-MS chromatogram for the pyrolysis of red meat at 400 ˚C (red line) and 500 ˚C (blue line)

While the concentration of most species as observed in Figure 4.2 decreased significantly at 500 ˚C, the concentration of indole appears to be comparably high. The oxygenated molecular products (phenols in particular) decompose at high temperatures to form mainly CO, water and other small molecules (Sharma and Hajaligol, 2003; Kibet *et al.*, 2012). The decrease in the concentration of 1,1'-biphenyl is anticipated because of the scission of the biphenyl bond leading to the formation of other by-products of combustion especially indole and other indole

derivatives in presence of nitrile radical pool in the combustion system. This is because meat is largely a protein. The dominance of nitro-PAHs and indole derivatives during high temperature cooking of meat is not unusual. At high temperatures, many complex reactions occur including intermediates, radical formation, and new products of aromatic nature (Kibet *et al.*, 2013). Long chain molecular products were also detected in significantly high concentrations and their structures are shown in Figure 4.3. Nevertheless, they are believed to be toxic due to their high molecular masses and the possibility of covalently bonding to biological structures such as DNA and lipids to cause severe health problems (Glover and Schumacher, 2016). Furthermore, these long chain compounds can transform to free radicals at high temperatures and consequently, their potency cannot be ignored.

Table 4.1: Major molecular toxins determined from this work

No.	Name of Molecular Toxin	Molecular Structure	Retention Time (mins)	Molar mass (g/mol)
a	Phenol		9.42	94.04
b	Indole		13.26	117.06
c	2,3-dimethylhydroquinone		16.14	153.20
d	2-(ethyl thio)phenol		18.86	154.05
e	1,1’ –biphenyl		19.12	154.08
f.	2-ethenylnaphthalene		19.68	154.08
g.	2-(1-methyl)quinoline		20.27	171.10

The compounds presented in Figure 4.3 are very important components of meat and were tentatively identified from the NIST and enhanced data libraries. These compounds can easily be correlated with the compounds found in a meat matrix.

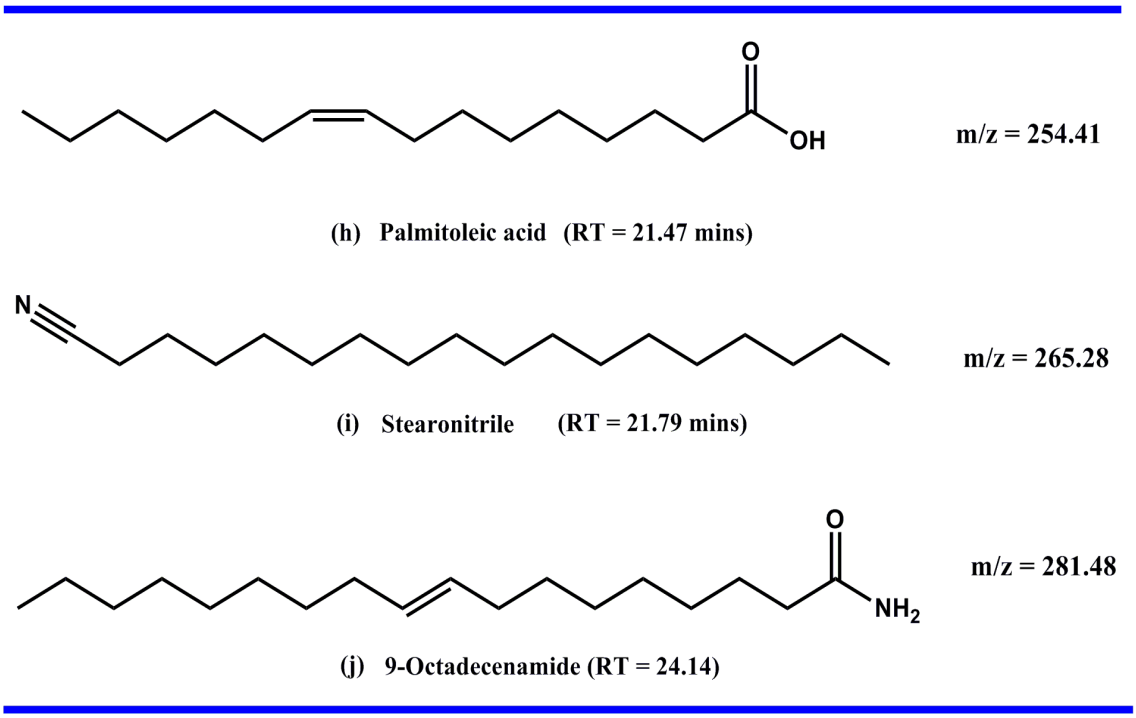


Figure 4.3: Long chain molecular compounds detected from the pyrolysis of goat meat

4.2 Decomposition profile of red meat

The decomposition characteristics of red meat over the temperature range of 300 -525 ˚C gave interesting results (Figure 4.4). The initial sharp decrease in % char to ~ 60% at 300 ˚C was very remarkable and may be attributed to high mass loss of water and other volatiles in the meat sample. Significant mass loss was also registered between 300 ˚C and 450 ˚C (approximately 36% mass loss). This was consistent with other results of biomass pyrolysis which indicate the higher mass loss of biomass pyrolysis occurs in this temperature region (Sharma and Hajaligol, 2003).

The mass loss between 350 and 450 ˚C was ~25%. This region coincides with the highest release of molecular toxins. Thus, by 450 ˚C most organic compounds will have been formed so that any further increase in temperature results in sharp decrease in the pyrolysis products (Figure 4.1).

These results corroborate previous data reported in literature on biomass pyrolysis (Kibet *et al.*, 2012; Kibet *et al.*, 2015).

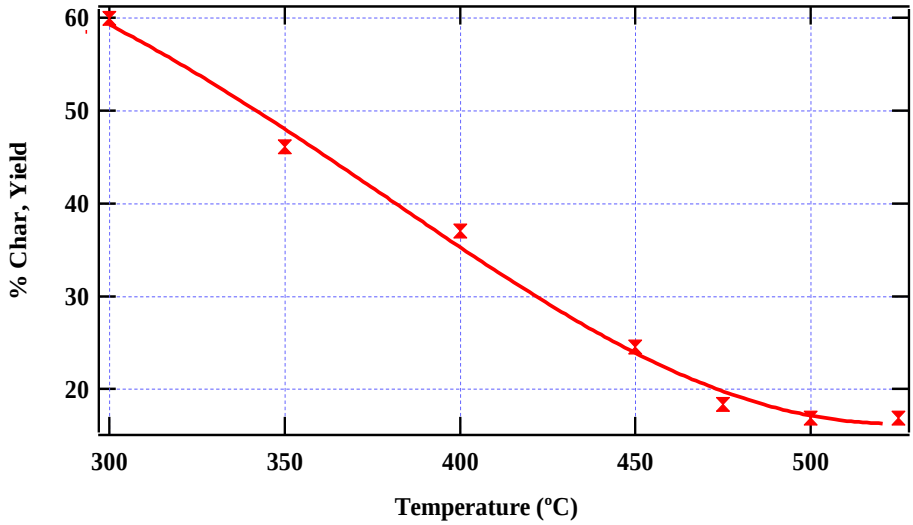


Figure 4.4: Percentage (%) yield of char from pyrolysis of goat meat.

The lowest mass loss was recorded at 525 ˚C ($\approx 17\%$). Between 500 ˚C and 525 ˚C, the mass loss was fairly constant. Above this temperature zone, the char is largely aromatic and few oxygenated compounds are often formed (Kibet *et al.*, 2012).

4.3 Characterization of Particulate emissions from high temperature cooking of goat meat

At an associated magnification of x 400, the size of particulate matter from cooked meat at temperatures of 500 ˚C and 700 ˚C were significantly different. At 500 ˚C, the particle size of emissions were larger ~ 7.72 ± 0.61µm (Figure 4.5) as compared to the particles at 700 ˚C ~ 3.52 ± 0.31 µm (Figure 4.6). This implies that the higher the cooking temperature, the smaller the soot particles. Nevertheless, the particulate size from high temperature cooking of red meat falls well under PM_{10} classification of particulate matter. Therefore our results corroborate studies reported elsewhere in literature regarding particulate emissions from high temperature cooking of red meat (Kamens *et al.*, 1991). Particles of this size are known to cause respiratory ailments including lung cancer and bronchitis. They composed of mainly organics such as phenols, PAHs, and possibly HCAs since they originate from the thermal degradation of red meat (Buonanno *et al.*, 2009). In many countries, about 80-90% of people spend most of their times indoors thus,

exposure to harmful emissions as a consequence of high temperature cooking and other combustion sources is very high.

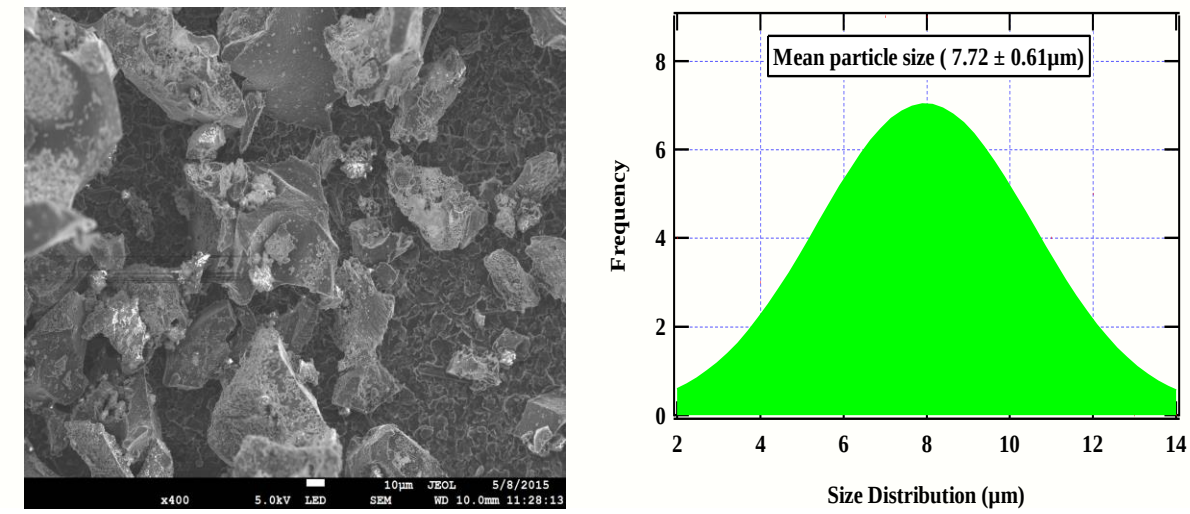


Figure 4.5: SEM image and particle size distribution (Gaussian green) of soot particles of meat at 500 ˚C.

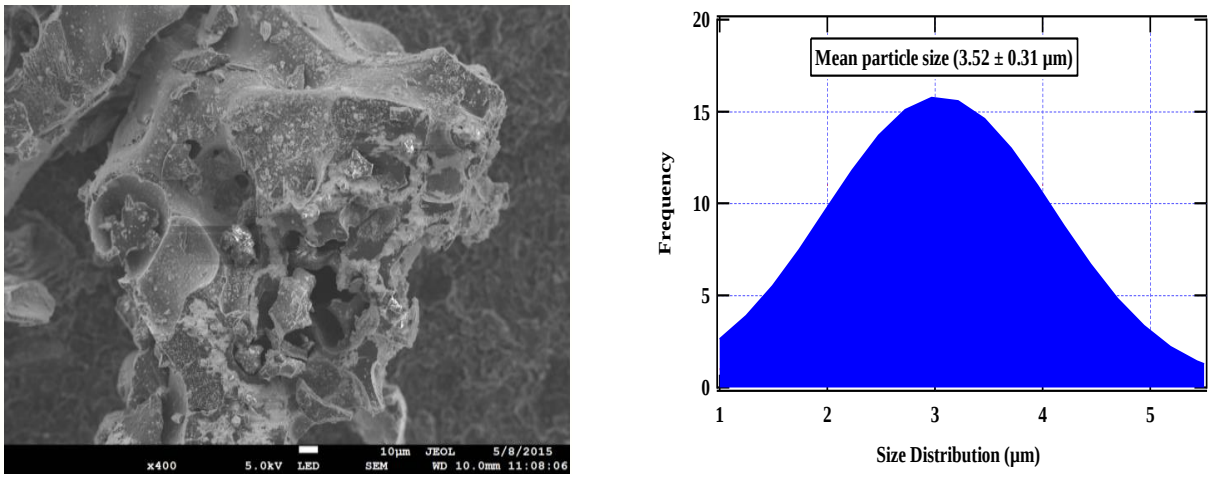


Figure 4.6: SEM image and particle size distribution (Gaussian blue) of particulate emissions of meat at 700 ˚C.

Statistically, as shown in table 4.2, there is a significant difference in the mean sizes of particulate emissions released at 500 ˚C and 700 ˚C at 95% confidence interval since the

calculated F-value = 37.53 is greater than the F-critical = 4.04, and P-value = 0.000 and thus less than 0.05 level of confidence.

Table 4.2: Mean sizes of particulate emissions formed at 500 and 700 ˚C

Temperature (˚C)	No of particles (N)	Mean sizes (µm)	Groupings
500	50	7.72±0.61	A
700	50	3.52±0.31	B

4.4 Formation kinetics of 2-(ethylthio)phenol from high temperature cooking

The Formation kinetics for 2-(ethylthio)phenol has been reported in this study as a representation of the formation kinetics of other molecular toxins. Moreover, 2-(ethylthio)phenol is less reported in literature hence its chemistry is necessary. A plot of ln c as a function of cooking time yielded a straight line with a slope of - 0.06426 (Figure 4.7) from which the formation rate constant of 2-(ethylthio)phenol (0.064 min^{-1}) was calculated.

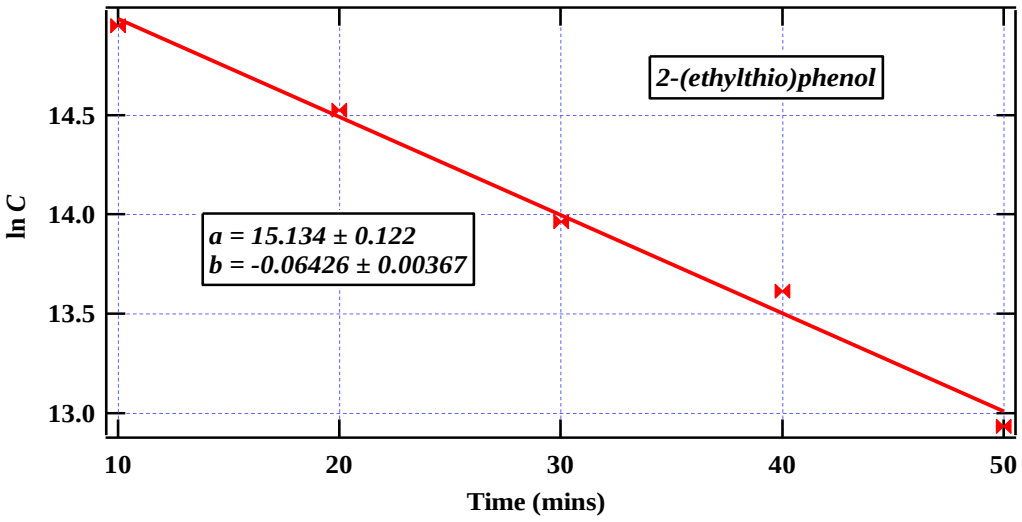


Figure 4.7: The formation kinetics of 2-(ethylthio)phenol from high temperature cooking of goat meat

The plot (Figure 4.7) was shown to be consistent with first order reaction kinetics and effectively obeyed the first order rate law. The original amount of 2-(ethylthio)phenol as determined from the y-intercept of Figure 4.7 was found to be 3.34 x 10^6 GC-Area counts and was remarkably close to that obtained from experimental modeling ~ 2.6 x 10^6 GC-Area counts. This suggests

both experimental modeling kinetics almost reproduced the concentration of 2-(ethylthio)phenol at the maximum release as presented in Figure 4.1. The decrease of 2-(ethylthio)phenol, with cooking times (Figure 4.7) was contrary to expectation that the accumulation of molecular products should increase with increase in cooking time. This can be attributed to the fact that shorter residence times impede side reactions whereas longer residence times led to radical formation, recombination, and pyrosynthesis of new by-products (Kibet *et al.*, 2012, 2013). Thus, these processes may decrease the yields of the parent compound, 2-(ethylthio)phenol.

4.5 Destruction kinetics of selected molecular toxins

This section will examine fundamentally the destruction kinetics of major combustion by-products of high temperature cooking. Accordingly, the destruction kinetics revealed that 2,3-dimethylhydroquinone, 2-(ethylthio)phenol, and indole occurred with respective activation energy barriers of 263.10, 283.38, and 315.76 kJmol^{-1} (Table 4.3). The destruction of indole proceeded with not only a high activation energy barrier but also a high collision frequency (Arrhenius constant, A). This high energy barrier is attributed to the π-π interactions which stabilize the indole molecule. The destruction energy barriers of 2,3-dimethylhydroquinone and 2-(ethylthio)phenol were significantly low and comparable with the destruction energies of other biomass components such as cellulose (Baker *et al.*, 2006; White *et al.*, 2011; Wang *et al.*, 2012). However, the destruction kinetics of biomass components from various materials may be different due to complex heterogeneous composition of biomass matrices. Therefore the activation energies of different species in the biomass may not necessarily be the same considering the fact that species in an organic matrix may act as catalysts and ultimately reduce or enhance the activation energy of a given compound in a complex biomass material such as meat (Burhenne *et al.*, 2013). Although a linear relationship between $\ln k$ and $1/T$ was considered, it is true that not all reactions will necessarily obey this relation. Therefore in order to estimate the Arrhenius dependent rate constants consistent with experimental rate constants then the modified Arrhenius rate law was applied (Equation 4.1).

$$k = AT^{n}e^{-\frac{Ea}{RT}} \qquad 4.1$$

Since the rate constant for a given temperature was determined experimentally and all the other parameters have been calculated, the value of n was determined from equation 4.1. For instance,

the value of n at 450˚C was determined and found to be -0.18, -0.18, and 0.09 for the destruction of indole, 2-(ethylthio)phenol, and 2,3-dimethylhydroquinone, respectively. It is also possible to use the same expression (equation 4.1) to determine the value of n at any temperature since the rate constant is temperature dependent.

Arrhenius plots for the destruction of molecular toxins from the meat sample under study are presented in Figures 4.8 and 4.9, *vide infra*.

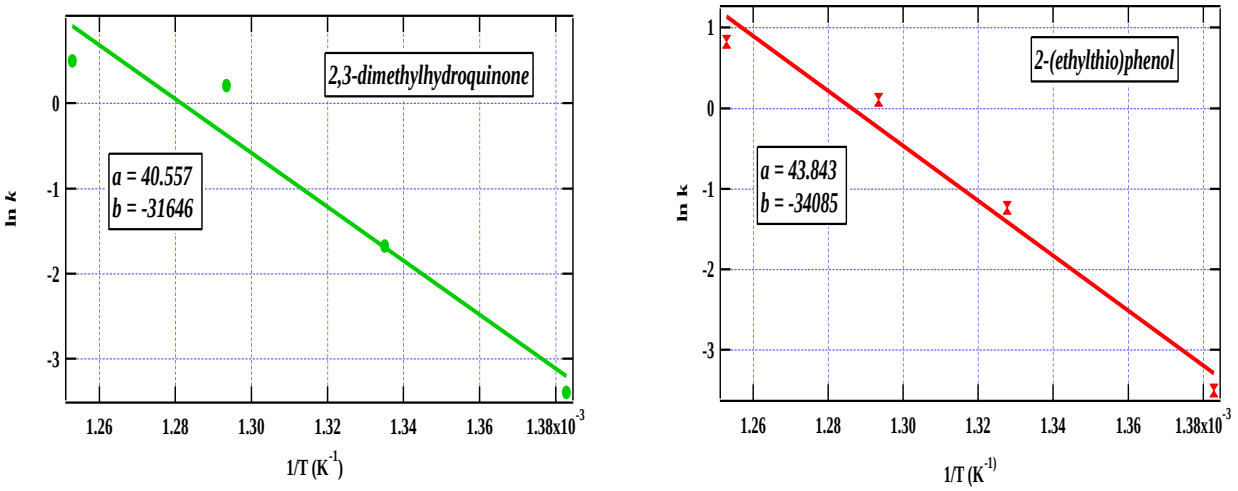


Figure 4.8: Arrhenius plots for the kinetic destruction of 2,3-dimethylhydroquinone and 2-(ethylthio)phenol at high temperatures

The destruction rate constant k_1 at 450˚C for indole, 2-(ethylthio)phenol, and 2,3-dimethylhydroquinone were found to be 0.006 s^{-1}, 0.011 s^{-1}, and 0.022 s^{-1} respectively. The rate constants were in the approximate ratios 1:2:4 implying that at 723 K, the rate of destruction for 2-(ethylthio)phenol was about twice the rate of destruction of indole. Using the same argument, it is evident that the destruction for 2,3-dimethylhydroquinone is approximately four times the rate of destruction of indole. Nevertheless, at the highest pyrolysis temperature 525˚C, the corresponding rates of destruction for indole, 2-(ethylthio)phenol, and 2,3-dimethylhydroquinone were 1.19 s^{-1}, 1.42 s^{-1}, and 1.35 s^{-1}. This shows that, as the temperature increases the rates of destruction also increases. The average rates of destruction for the compounds under investigation (indole, 2-(ethylthio)phenol, and 2,3-dimethylhydroquinine) were 0.94 s^{-1}, 1.31 s^{-1}, and 0.87 s^{-1}, respectively. This suggests that individual temperatures give a higher resolution on the destruction of the compound than the entire temperature range.

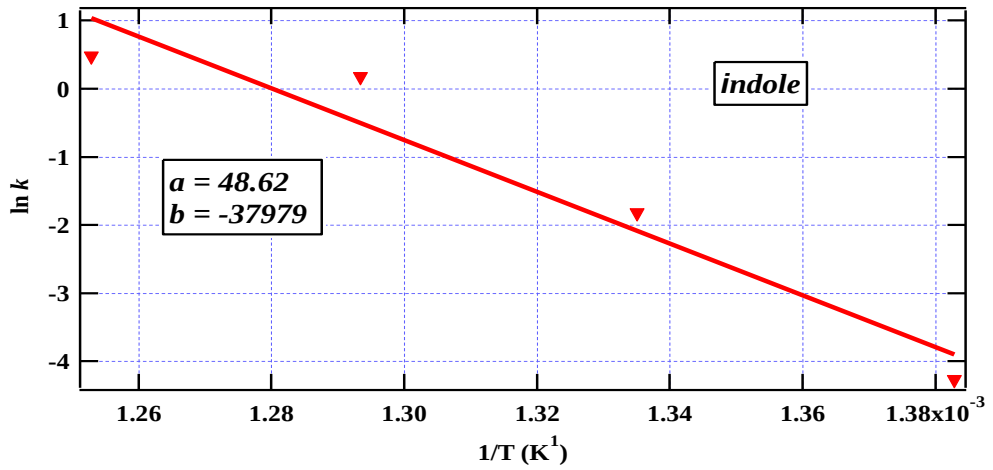


Figure 4.9: The destruction kinetics of indole at high temperatures (450-525 ˚C)

Slope = - $\frac{Ea}{R}$, therefore activation energy (Ea) was obtained from; Ea= slope×R

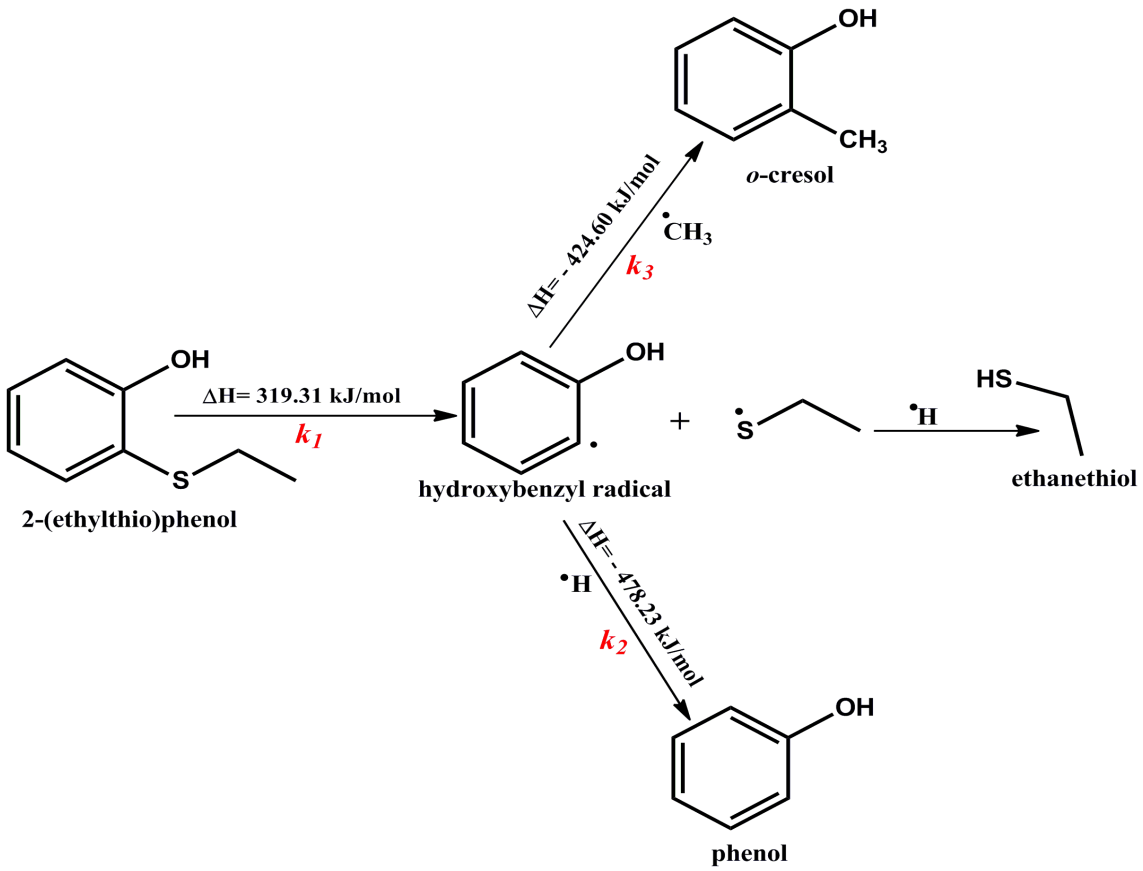
Table 4.3 presents the Arrhenius parameters from the destruction kinetics of molecular toxins under investigation (Activation energies and Arrhenius factors). Whereas the activation energies are comparably close, the pre-exponential factors (A) for the compounds under study are significantly different. For instance, the ratio of pre-exponential factors for 2,3-dimethylhydroquinone, 2-(ethylthio)phenol, indole were in the order, 1:27:3200. The ratios are given in approximate whole numbers and it is clear the collision rate during the thermal destruction of indole is very high in comparison with the collision rate for the destruction of other molecular toxins investigated in this work.

Table 4.3: The Arrhenius parameters for the destruction of bio-hazardous combustion by-products from high temperature cooking of goat meat.

Compound	**Ea (kJmol^{-1})**	**A (s^{-1})**
2,3-dimethylhydroquinone	263.10	4.11 x 10^{17}
2-(ethylthio)phenol	283.38	1.10 x 10^{19}
Indole	315.76	1.30 x 10^{21}

4.6 Proposed mechanistic pathways for the destruction of 2-(ethylthio)phenol

The proposed mechanism for the formation of phenol from 2-(ethylthio)phenol is presented in scheme 4.1, *vide infra*. All the energies shown in the scheme were estimated quantum mechanically using the Density Functional Theory (DFT). Clearly, there are two major competing reaction channels; (i) the formation of phenol with an enthalpic barrier of -478.23 kJmol^{-1} and (ii) the formation of *o*-cresol which occurs with an energy barrier of - 424.60 kJmol^{-1}.



Scheme 4.1: Mechanistic degradation pathways of 2-(ethylthio)phenol

Evidently, by substituting the original concentration of 2-(ethylthio)phenol (2.60 x 10^6 GC-Area counts) and the maximum concentration of the product, in this case, phenol (8.39 x 10^5 GC-Area counts) into equation 3.13, *vide supra*, the value of k_2 was calculated and found to be 0.57 s^{-1}. This showed that the value of k_1, was two folds greater than k_2. This implies that free radicals

formation (2-hydroxybenzyl radical) proceeds faster than the formation of the neutral compound phenol. Consequently, since the rate constants k_1 and k_2 have been estimated, and the original value of 2-(ethylthio)phenol is known, then the concentration of the intermediate, 2-hydroxybenzyl radical can be computed from equation 3.11 from which the concentration of 2-hydroxybenzyl radical was found to be 1.14 x 10^6 GC-Area counts. This value confirms the presence of free radicals from high temperatures cooking of red meat associated with health effects such as cell injury, aging, gouts, and cancer (Lobo *et al.*, 2010).

The sum of the concentrations of the intermediates and the proposed final product (phenol) was estimated at 1.98 x 10^6 GC-Area counts. This shows that only ~ 76% of 2-(ethylthio)phenol was converted to the final product (phenol). In a complex matrix such as meat not all 2-(ethylthio)phenol is converted to the intermediate and ultimately to the product. Some other side reactions compete during combustion processes leading to formation of other by-products. On the other hand, the rate constant k_3 was not calculated because *o*-cresol was not detected experimentally. The product distribution of phenol in the entire temperature range is presented in Figure 4.10.

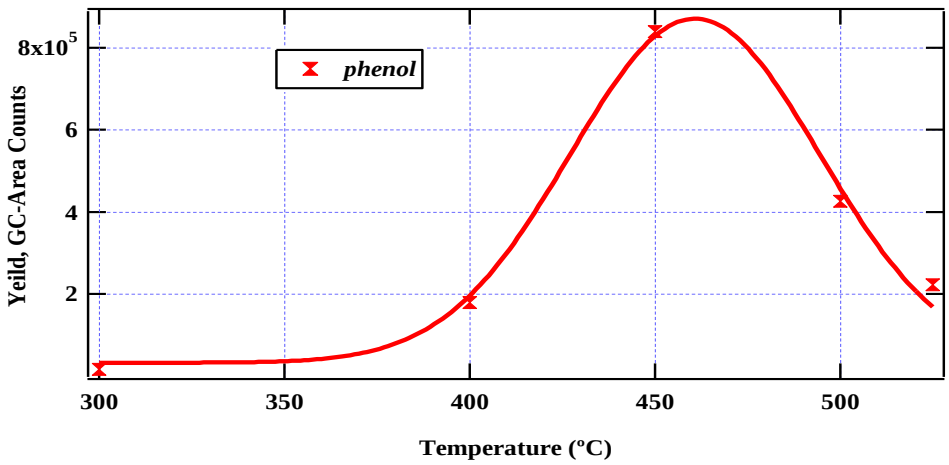


Figure 4.10: The product distribution of phenol from high temperature cooking of red meat

4.7 Computational modeling and toxicity index of 2-(ethylthio)phenol

The bond dissociation energy via k_1 and the bond formation energy via k_2 in scheme 4.1 *vide supra*, were estimated using the density function theory framework at the B3LYP energy functional in conjunction with 6-31G basis set. The bond dissociation energy for the scission of

C-S bond (cf. scheme 4.1) was found experimentally to be 283.38 $kJmol^{-1}$ whereas the theoretical value was found to be 319.31 $kJmol^{-1}$ as calculated using the most accurate DFT analytical gradient. Considering the fact that the pyrolysis of meat is highly heterogeneous as well as complex, the experimental results presented in this study on the energetics of 2-(ethylthio)phenol are acceptable.

Despite continuing improvements in formulating new DFT functionals with advanced predictive capabilities, the B3LYP functional retains its comparative accuracy in general applications to organic systems (Uddin *et al.*, 2012; Boese, 2015). The application of Chemissian software facilitated the construction of electron density contour maps and molecular orbitals from which the band gap between the HOMO and the LUMO of 2-(ethylthio)phenol was calculated and found to be - 5.298 eV as shown in Figure 4.11.

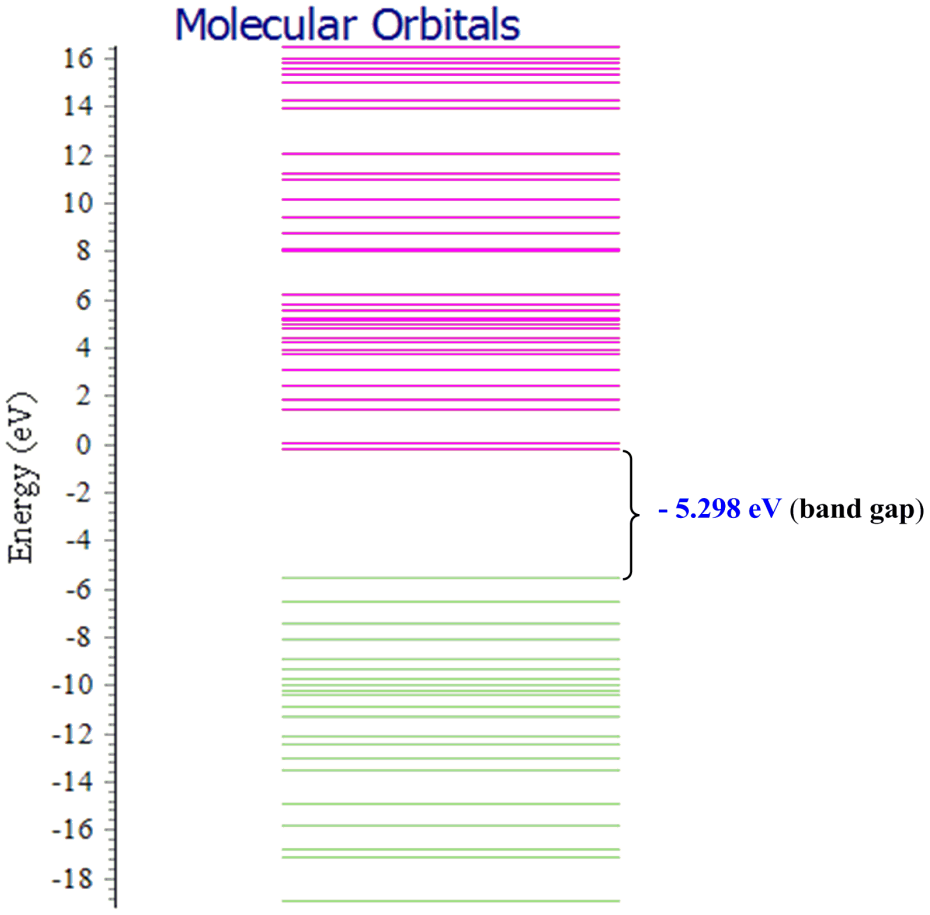


Figure 4.11: The HOMO-LUMO band gap for 2-(ethylthio)phenol determined using Chemissian

The electron density contours and molecular orbital for 2-(ethylthio)phenol are presented in Figures 4.12 and 4.13 respectively.

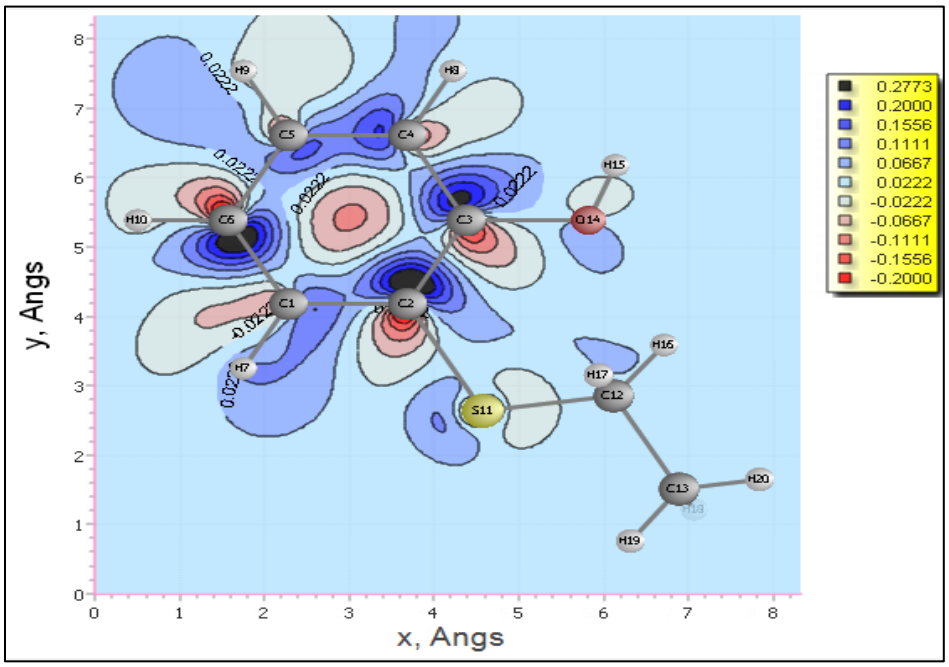


Figure 4.12: 2-D electron density map for 2-(ethylthio)phenol

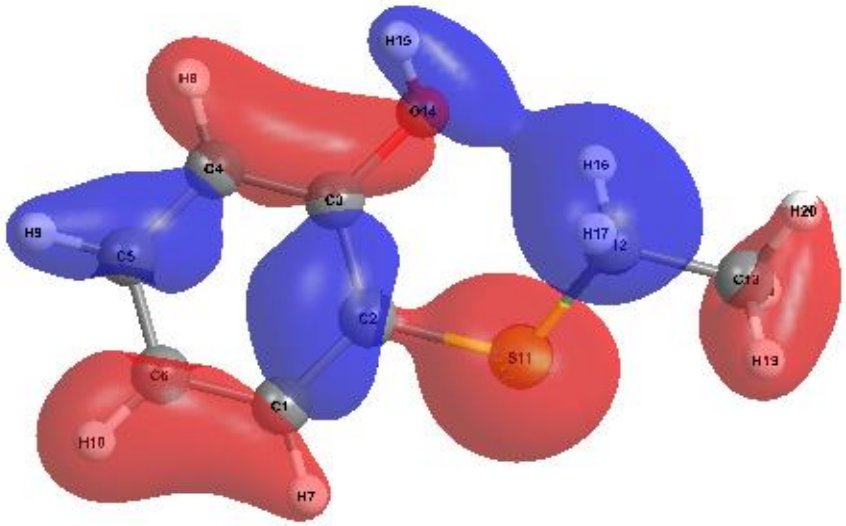


Figure 4.13: 3-D molecular orbital diagram showing electronic density for 2-(ethylthio)phenol at an isovalue of 0.02.

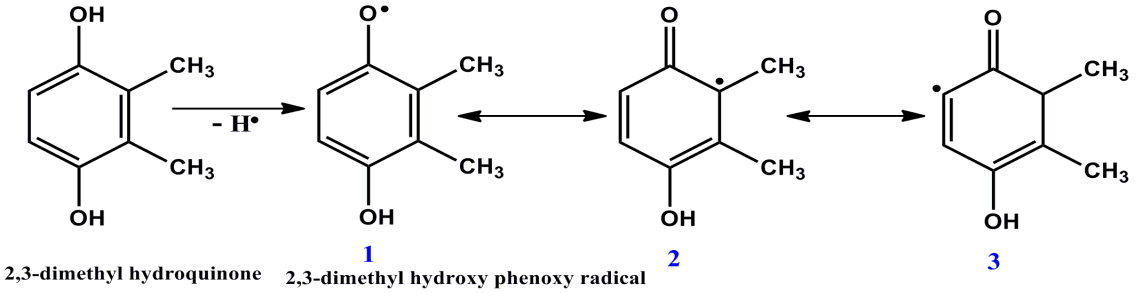
For organic molecules the electron density is rich in the most electronegative atoms and flow towards the less electronegative atoms. In the case of 2-(ethylthio)phenol the most positive electrostatic potentials are around the hydrogen atoms while the most electronegative potentials are around the electron rich benzene ring, oxygen, and to a less extent the sulphur atoms. Nonetheless, the electronegativity of the neighbouring molecules will have a significant bearing on the electron distribution. Therefore studies on molecular orbitals and electron density maps are critical towards understanding the reactivity of molecules especially frontier orbitals.

The toxicity indices for 2-(ethylthio)phenol, indole, and 2,3-dimethylhydroquinone were estimated using HyperChem computational platform and reported as log P = 0.26, log P=-0.24, and log P = - 0.15 respectively. The measure of toxicity is based on the octanol-water partition coefficient (Smith and Hansch, 2000; Carlsen *et al.*, 2008) and shows that 2-(ethylthio)phenol is about 1.8, indole is 0.58, while 2,3-dimethylhydroquinone is 0.71 times more soluble in octanol than in water hence these compounds are generally hydrophobic because of the intense carbon content in their structures. Nevertheless, hydrophobic compounds are known to cause toxicity by covalently bonding with lipids and other biological structures to release harmful metabolites such as quinones (Bolton *et al.*, 2000; Hirakawa *et al.*, 2002). Hydrophobic compounds have the potential to cross biological barriers containing lipids, DNA, and microsomal cells causing serious cell injury and ultimately cell impairment (Debnath *et al*., 1994; Smith and Hansch, 2000).

4.8 The mechanistic pathways for some selected molecular by-products of combustion

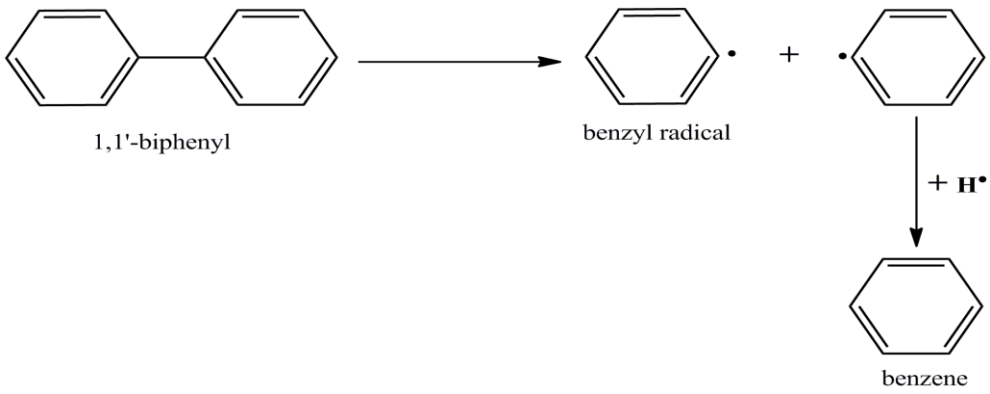
It can be noted from scheme 4.2 that 2,3-dimethyl hydroxy phenoxy radical (1) is at resonance with benzoquinone type radicals (2,3-dimethyl hydroxy-2-benzoquinone (2) and 2,3-dimethyl hydroxy-6-benzoquinone (3) radicals). Radicals 1, 2, and 3 are resonance hybrids. To investigate the energetics of these radicals, quantum chemical calculations were conducted using the density functional theory (DFT/B3LYP) in conjunction with 6-31G basis set. It was found that radicals 1 and 2 were formed via an exothermic energy barrier of -295.90 $kJmol^{-1}$. Formation of radical 3 nonetheless was predicted to be highly exothermic (-537.21 kJ/mol^{-1}). This enthalpy is ~ 1.8 times the energy observed for the formation of radicals 1 and 2. Therefore, the formation of radical 3 is accompanied by a highly exothermic process. The conversion of radical 1 to radical 2 is barrierless (i.e. the change in enthalpy for the conversion of radical 1 to radical 2 is ~ 0 $kJmol^{-}$

[1]). The characteristic behaviour of phenoxy radical and its associated health effects is documented in literature and will not be the subject of further investigation in this study (Khachatryan *et al.*, 2010a; Khachatryan *et al.*, 2010b; Kibet *et al.*, 2012).



Scheme 4.2: Mechanistic formation of radicals from 2,3-dimethyl hydroquinone

The carcinogenicity of indole is reported in literature (Sugimura *et al.*, 2004; Majima and Toyokuni, 2012). Nitrogen-containing compounds are present in high temperature cooking ($\geq 120^{\circ}C$) processes such as frying, baking and grilling (Maga, 1981). High temperature pyrolysis $\geq 500^{\circ}C$ for various food materials has been shown to result in indole formation and other nitrogen containing compounds (Maga, 1981; Duncan *et al.*, 2009). On the other hand, 1,1'-biphenyl can undergo rapture of the biphenyl bond (C-C bond) at high temperatures, resulting to toxic benzyl radicals which can immediately convert to benzene in the presence of hydrogen radical 'pool'. Scheme 4.3 shows how benzyl radical can be formed from 1,1'-biphenyl. It is reported in literature that benzene can cause hematopoietic disorders, leukemia as well as cancer (Junjie *et al.*, 2014) and hence may pose potential risks to both environmental and public health.



Scheme 4.3: Mechanistic formation of benzyl radical and benzene from 1,1'-biphenyl

From a theoretical stand point using quantum mechanical calculations (DFT/B3LYP) analytical framework and 6-31G basis set, the scission of the biphenyl bond is accompanied by an endothermicity of 461.80 $kJmol^{-1}$ whereas the formation of benzene from benzyl radical proceeds with an enthalpy change of -466.53 $kJmol^{-1}$. This suggests that at high temperatures, benzyl radical is formed in the gas phase although in the presence of hydrogen radicals, it can easily be converted to the well-established carcinogen, benzene (Snyder *et al.*, 1993) which was detected in low amounts in this work.

4.9 Toxicological implications of molecular products and emissions from combustion

It is evident from the compounds detected in this study that emissions from high temperature cooking of red meat are a potential risk to human health. For instance, phenols (phenol and 2,3-dimethyl hydroquinone) provoke mutagenesis and carcinogenesis towards humans and other ecosystems through generation of organic radicals and reactive oxygen species (ROS) (Michalowicz and Duda, 2007). More importantly, phenol at high temperatures is capable of forming phenoxy radicals that are transient reactive species capable of reacting with biological tissues causing extensive cellular damage and eventually cancer (Khachatryan *et al.*, 2008; Kibet *et al.*, 2012). The compound 2,3-dimethyl hydroquinone can undergo hydrogen abstraction to form a benzoquinone type radical, usually considered extremely reactive and a potential cause for oxidative stress and possibly cardiac arrest (Khachatryan *et al.*, 2008; Kibet *et al.*, 2013). Following H abstraction, the resultant benzoquinone and phenoxy radicals exhibit electron deficient characteristics. The benzoquinone type radical is stabilized by electron donating groups (methyl groups) and thus it is believed to be an environmentally persistent free radical (Dellinger *et al.*, 2007; Khachatryan *et al.*, 2008). Such environmentally persistent free radicals can cause irreparable damage to the biological genetic make-up and consequently carcinogenesis and other cancer related ailments. Scheme 4.2 shows the mechanistic formation of some of the possible radicals from 2,3-dimethyl hydroquinone at high temperatures. Radicals produced during high temperature cooking of food materials are therefore very reactive and capable of causing oxidative stress, cancers, and coronary health problems including cardiac arrest (Dellinger *et al.*, 2007; Kibet *et al.*, 2013).

Epidemiological studies have revealed that exposure to particles of aerodynamic diameter of $PM_{2.5}$ and PM_{10} enhances lung cancer, morbidity, and cardiopulmonary death (Junjie *et al.*,

2014). The aerodynamics of airborne particulate matter and the deposition characteristics in the human lung has shown that airborne particulate matter with an aerodynamic average diameter less than 2.5 μm ($PM_{2.5}$) is the fraction of the particles with the largest impact in human health (Squadrito *et al.*, 2001). The particulate matter at 700 ˚C in this study however is far much smaller and can be inhaled deeper resulting to severe lung damage and serious respiratory problems including whizzing of the lungs. Despite intense interest in the toxicity of $PM_{2.5}$, the mechanism by which it causes ill health is poorly understood (Dellinger *et al.*, 2001). Combustion particulate matter is known to contain free radicals and thus airborne fine particles from combustion especially $PM_{2.5}$ may contain radicals which are injurious to human health (Dellinger *et al.*, 2000; Dellinger *et al.*, 2001). Ultrafine PM (< 0.1 μm) is an important sub-fraction of fine PM and has potentially unique toxicological properties (Mirowsky *et al.*, 2013). The fine particles provide a carrier for deposit of the radicals deep in the human respiratory tract. These radicals consequently initiate immune system responses that can activate the production of more radicals as well as other species that can cause damage to DNA and/or induce damage to the respiratory airway (Dellinger *et al.*, 2000). Nonetheless, the health effects of airborne fine particles are still a matter of critical scientific debate (Squadrito *et al.*, 2001). The particulates explored in this investigation from high temperature cooking of red meat fall under the $PM_{2.5}$ and PM_{10} categories. Such classes of particulates are well known candidates for various disease burdens in the respiratory landscape. Therefore chefs in meat cooking joints and homes may be at serious risks of contracting emphysema, and lung diseases (alveoli and microphage damage).

CHAPTER FIVE

CONCLUSIONS AND RECOMENDATIONS

5.1 Conclusions

This work has revealed various classes of bio-hazardous by-products of high temperature pyrolysis of goat meat. In particular, the major by-products included phenolics (phenol, 2-(ethylthio)phenol, and 2,3-dimethyhydroquinone), PAHs (2-ethenylnapthalene and 1,1'-biphenyl), and nitro-PAHs of the heterocyclic amine group (indole and 2-(1-methyl)quinoline), and long chain molecular toxins such as stearonitrile.

The molecular toxins investigated have exhibited various kinetic characteristics possibly because of their heterogeneous kinetic behaviour in complex biomass materials such as meat. The concentration of the intermediate (2-hydroxybenzyl radical) has been estimated from the kinetic modeling of 2-(ethylthio)phenol. This is remarkable because the concentrations of intermediates in complex reaction systems such as biomass are usually difficult to determine experimentally. The kinetic parameters presented in this study have been derived entirely from experimental work. DFT calculations and experimental predictions for the energetics of 2-(ethylthio)phenol are very close despite the complex nature of meat pyrolysis.

The particulate nature of soot from combustion of red meat suggests that at 500 ˚C the particulate characteristics are approximately PM_{10} while the particulate size at 700 ˚C is ~ $PM_{2.5}$. Therefore the size of emissions from the pyrolysis of goat meat obtained from this study is respirable and can cause serious respiratory problems such as whizzing of the lungs. $PM_{2.5}$ emissions for instance can be inhaled deeper and hence capable of penetrating into the alveoli tissues to cause oxidative stress and other respiratory problems. On the other hand PM_{10} are deposited significantly on the lung surface due to their large size and may be precursors for the growth of tumours and ultimately cancer.

5.2 Recommendations

This investigation has thoroughly examined the evolution of major organic toxins and particulate emissions from high temperature pyrolysis of goat meat. Nonetheless, the following recommendations are necessary.

1. There is need to extend this investigation to various types of meats such as pork, chicken, and beef, and therefore expand the current body of knowledge.
2. This study recommends a beneficial ‘cooking’ temperature of ≤ 300 ˚C based on the results reported in this work.
3. The formation kinetics of other major pyrolysis products of high temperature cooking such as phenol, indole, and 1,1’-biphenyl should be explored in future studies.

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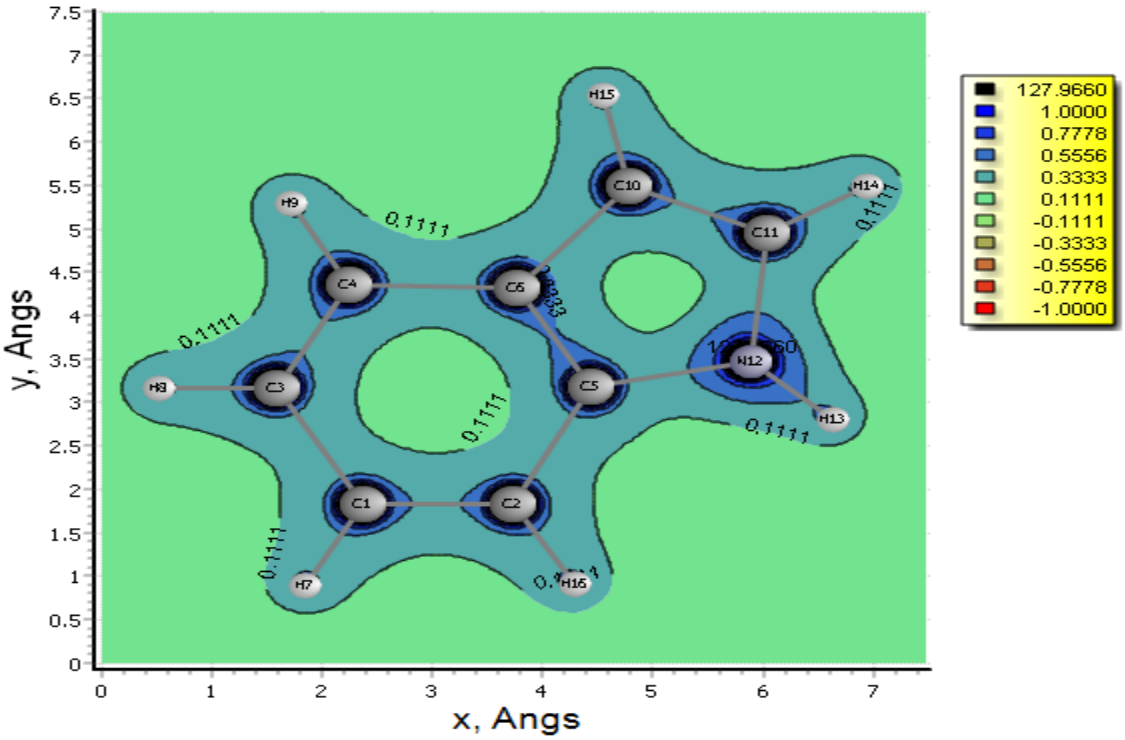
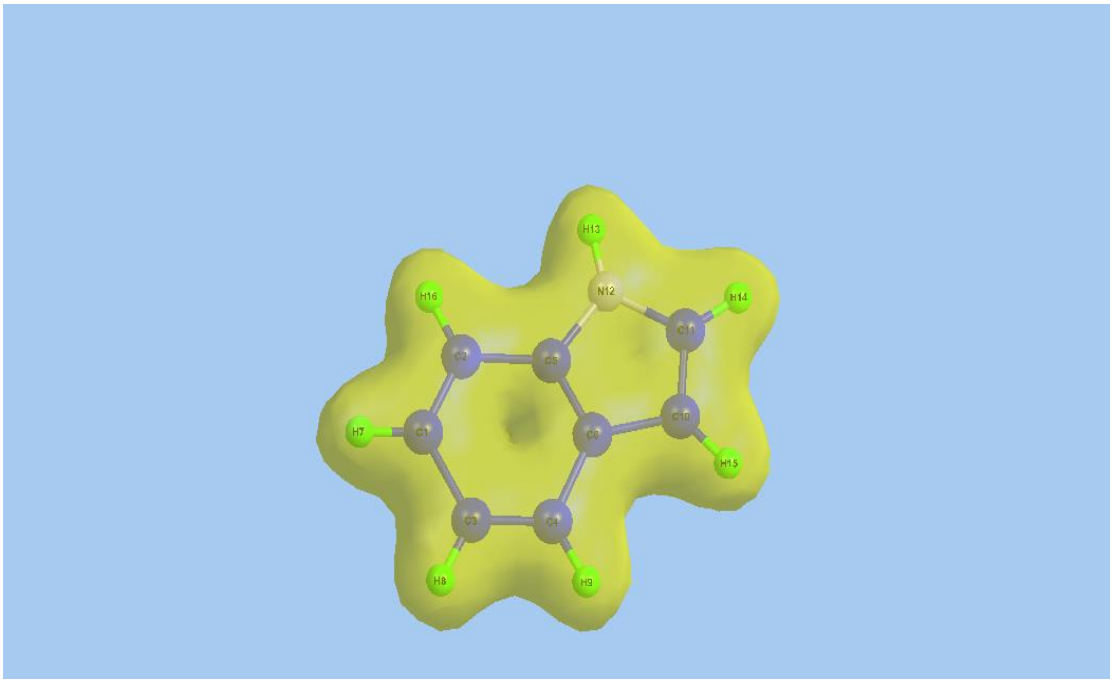
APPENDICES

Appendix 1A: 2-D electron density contour map for indole calculated using Chemissian



Appendix 1B: 3-D electron density map for Indole predicted using Chemissian



Appendix 2: The thermochemistry output file from Gaussian '09 computational platform

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- Thermochemistry -

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Atom 1 has atomic number 6 and mass 12.00000

Atom 2 has atomic number 6 and mass 12.00000

Atom 3 has atomic number 16 and mass 31.97207

Atom 4 has atomic number 1 and mass 1.00783

Atom 5 has atomic number 1 and mass 1.00783

Atom 6 has atomic number 1 and mass 1.00783

Atom 7 has atomic number 1 and mass 1.00783

Atom 8 has atomic number 1 and mass 1.00783

Molecular mass: 61.01120 amu.

Principal axes and moments of inertia in atomic units:

	1	2	3
EIGENVALUES --	160.50410	322.57011	483.07421
X	0.84511	-0.53459	0.00000
Y	0.53459	0.84511	0.00000
Z	0.00000	0.00000	1.00000

This molecule is an asymmetric top.

Rotational symmetry number 1.

Rotational temperatures (Kelvin) 0.53964 0.26851 0.17930

Rotational constants (GHZ): 11.24421 5.59488 3.73595

4 imaginary frequencies ignored.

Zero-point vibrational energy 138262.7 (Joules/Mol)

33.04557 (Kcal/Mol)

Warning -- explicit consideration of 2 degrees of freedom as

vibrations may cause significant error

Vibrational temperatures: 66.41 503.59 898.76 922.06 1354.07

(Kelvin) 1417.75 1490.27 1880.28 2097.95 2383.14

4551.70 4577.82 4697.58 6416.93

Zero-point correction = 0.052661 (Hartree/Particle)

Thermal correction to Energy = 0.057120

Thermal correction to Enthalpy = 0.058065

Thermal correction to Gibbs Free Energy = 0.024030

Sum of electronic and zero-point Energies = - 477.223199

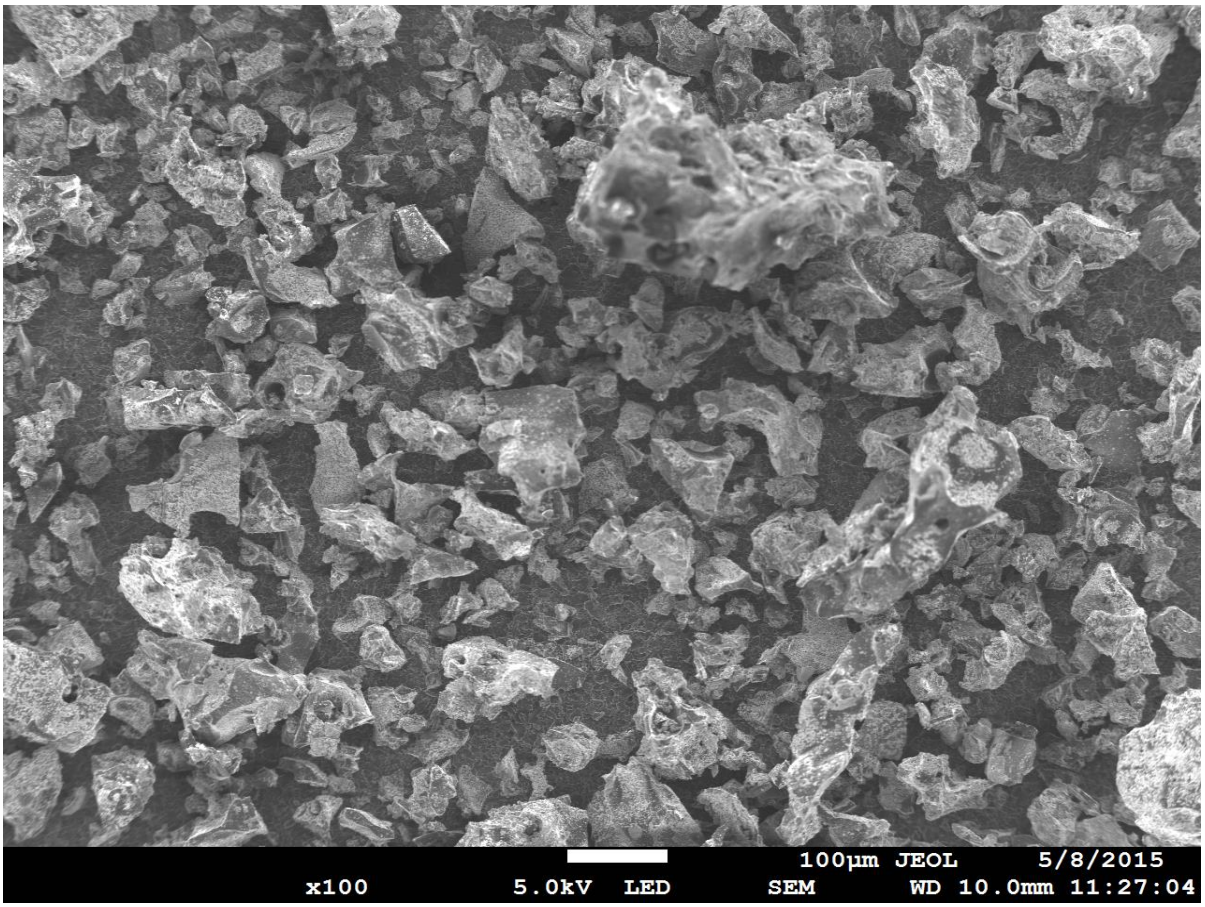
Sum of electronic and thermal Energies = - 477.218740

Sum of electronic and thermal Enthalpies = -477.217795

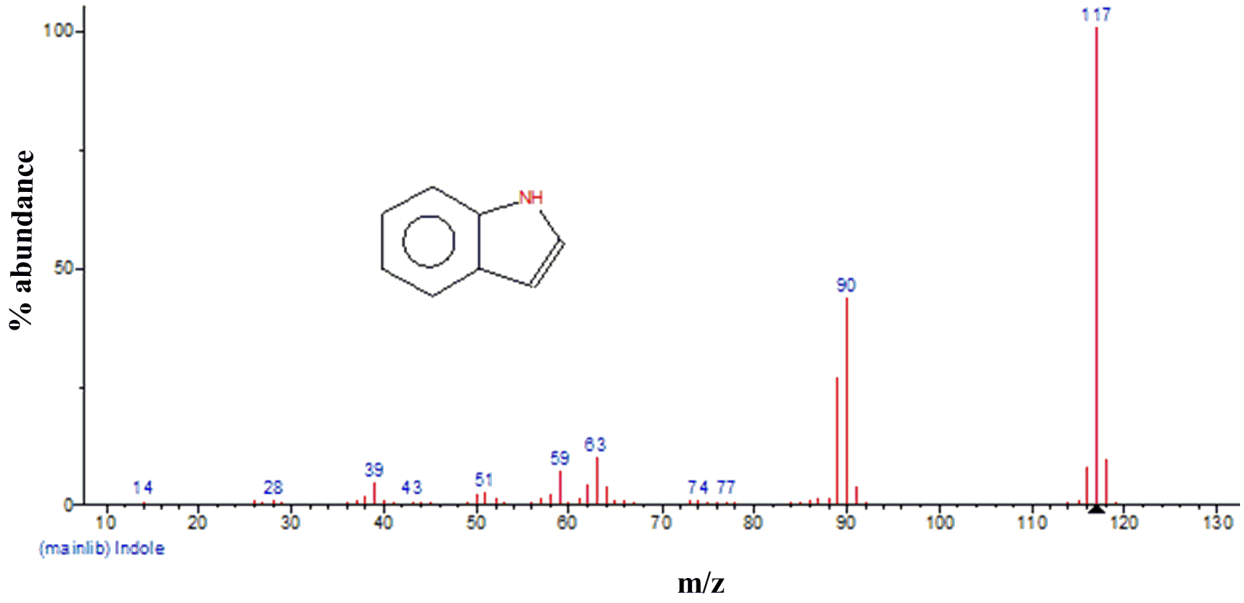
Sum of electronic and thermal Free Energies = -477.251830

	E (Thermal) KCal/Mol	CV Cal/Mol-Kelvin	S Cal/Mol-Kelvin
Total	35.844	12.896	71.632
Electronic	0.000	0.000	1.377
Translational	0.889	2.981	38.245
Rotational	0.889	2.981	24.729
Vibrational	34.066	6.935	7.281
Vibration 1	0.595	1.979	4.976
Vibration 2	0.727	1.575	1.166

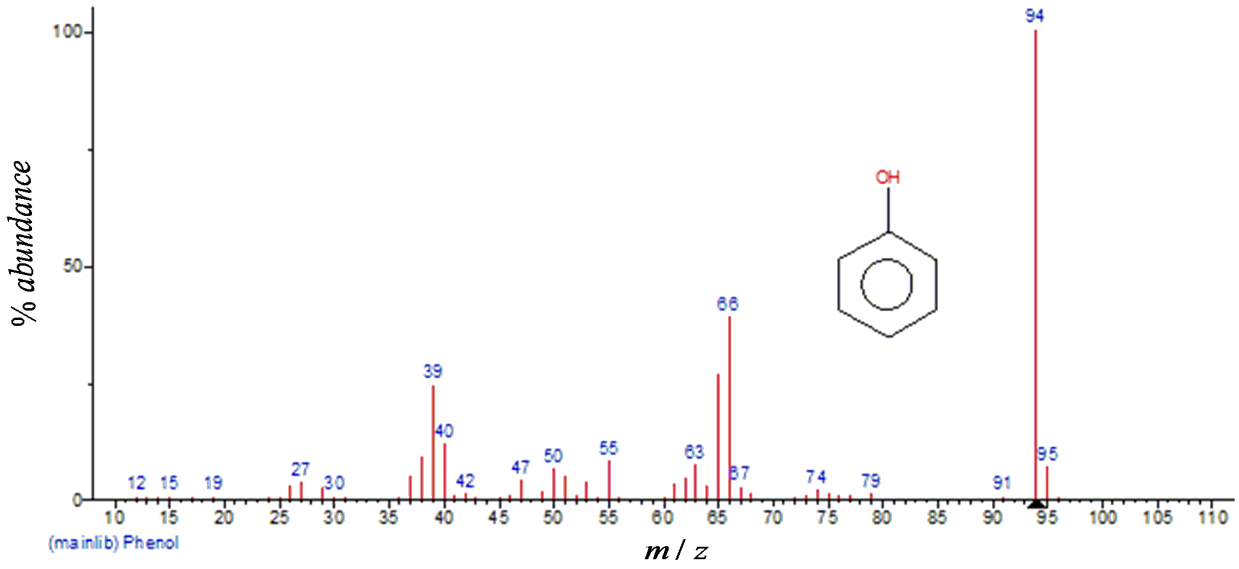
Appendix 3: A Sample of SEM micrographs at 700 ˚C pyrolysis of goat meat at an associated magnification of x 100



Appendix 4A: MS-Fragmentation pattern of indole using Electron Impact Ionization (EI)



Appendix 4B: MS-Fragmentation pattern of phenol using Electron Impact Ionization (EI)



Appendix 5: A Minitab software *vers. 16* output file for one way ANOVA: Diameter vs. no of particles

Source	DF	SS	MS	F	P
Temp	1	441.0	441.0	37.53	0.000
Error	98	1151.6	11.8		
Total	99	1592.6			

S = 3.428 R-Sq = 27.69% R-Sq(adj) = 26.95%

Individual 95% CIs For Mean Based on

Pooled StDev

Level N Mean StDev ----+---------+---------+---------+-----

sm1 50 7.720 0.61 (-----*-----)

sm2 50 3.520 0.31 (-----*-----)

----+---------+---------+---------+-----

3.2 4.8 6.4 8.0

Pooled StDev = 3.428

Grouping Information Using Fisher Method

No of

Particles N Mean Grouping

500 ˚C 50 7.720 A

700 ˚C 50 3.520 B

Means that do not share a letter are significantly different.

Fisher 95% Individual Confidence Intervals

All Pairwise Comparisons among Levels of No of Particles

Simultaneous confidence level = 95.00%

No of Particles = sm1 subtracted from:

No of

Particles Lower Center Upper --------+---------+---------+---------+-

sm2 -5.561 -4.200 -2.839 (------*------)

--------+---------+---------+---------+-

-4.0 -2.0 0.0 2.0