KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

NITRITE ASSESSMENT OF PROCESSED MEAT PRODUCTS COMMONLY CONSUMED IN GHANA

A THESIS SUBMITED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

BY

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MAY, 2017

DECLARATION

I declare that this submission is the result of my own research and thus does not contain any previously published material except for some information which the source for each one has been stated clearly.

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DEDICATION

This work is sincerely dedicated to the almighty God; my dearest husband Mr. Godfred Duah, my children Emmanuel Osei Duah, Eric Twumi Duah and Christabel Denkyi Duah.

ACKNOWLEDGEMENTS

To God be the glory, great things has He done. God has been merciful and gracious unto me throughout my life and more especially through this course, I am forever grateful unto Him. My profound gratitude goes to my supervisor Dr. Jacob Agbenorhevi, as he was cooperative and supportive and also to Mr Isaac W. Ofosu for all the help to bring this work to completion. I am indebted to my family especially my mother Mrs. Cecilia Kontoh and my father late Nana Ohene Kontoh who took care of my kids during the course. I will love to express my utmost gratitude to all my siblings for their prayers, support, love, and kindness to me during my study.

ABSTRACT

Sodium and potassium salts of nitrite are used in meat products as preservative, antioxidant and colour fixative. However, high levels of these chemicals pose risks such as toxicity and carcinogenic effects due to the formation of nitroso compounds originated from nitrite sources. The present study was conducted to assess the nitrite residual levels and to evaluate the health risk of consumers from exposure of nitrite in commonly consumed processed meat products (sausage, corned beef and bacon) sold on the Ghanaian market. A total of 300 questionnaires were administered to all categories of ages in the study population in the Ayawaso West Sub Metro, Accra. Participants were made to fill out the 24 h food frequency questionnaire. A total of Fifty (50) samples of different types and brands of processed meat products were randomly selected and analyzed for nitrite residues by spectrophotometric method. The mean nitrite content in the samples was 139.85 mg/kg and the mean daily intake estimated at 114.89 mg/kg/day. Significant to the findings of this research is that the nitrite levels exceed the WHO/EU recommended levels of 125mg/kg in processed meat. First order Monte Carlo simulation at 10,000 iterations estimated chronic daily intake of nitrite as 5.05 mg/kg/day. Subsequently, the average risk of consumption of processed meat within the limits of reference dose of nitrite (0.33 mg/Bw/day) for the studied population was estimated at 15.65. This means that, the risk quotient or hazard quotient was far greater than 1, which was indicative of adverse health effect to the consumers.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Meat consumption in developing countries has increased tremendously and the rising demand due to the fast progression of urbanization and the tendency among city dwellers to spend more on food than the lower income earning rural population.

The human desire for meat consumption has biological roots. In ancient times meat was clearly preferred, consequently time and physical efforts were invested to obtain it, basically through hunting and this enable the physical and mental development of humankind even though some people prefer meatless diets (Heinz and Hautzinger, 2007)

Meat remains the major source of protein than plant foods (except soy bean products). In meat, the essential amino acids – the organic acids that are integral components of proteins and which cannot be synthesized in the human organism – are made available in well balanced proportions and concentrations. As well, plant food has no Vitamin B_{12}; thus animal food is indispensable for children to establish B_{12} deposits. Animal food, in particular meat, is rich in iron, which is of utmost importance to prevent anemia, especially in children and pregnant women (Heinz and Hautzinger, 2007).

Globally meat production has increase to nearly 320 million tons by 2016 compare to annual production of 267 million tons in 2006 with developing countries accounting for the increase in production of over 50 million tons. To meet this growing demand meat processing plays a pivotal role (Heinz and Hautzinger, 2007). Meat processing involves the manufacturing of meat products from muscle meat, animal fat and certain non-meat additives. Additives are used to enhance product flavour and appearance and also increase product volume.

In meat processing, different meat product can be produced both from edible livestock parts and those that are not sold in fresh meat markets (e.g. muscle trimmings, bone scraps, skin parts or certain internal Organ and whole blood) into the food chain as valuable protein-rich ingredients. Due to the fact that world consumption of meat has continuously increased because meat remain the major source of animal protein for human and the annual per capita consumption increased by 2.6 fold in 2000 and will increase by 3.7 fold by 2030 compared to that of 1960s. The breakdown of valuable content of meat (protein, fat, carbohydrate) leads to off flavors development, and slime formation, which make it undesirable for human consumption (Dave, 2011).

Due to globalization and population growth, there is the need to minimize spoilage of meat in order to preserve, maintain nutritional value, texture and flavor. Energy intensive freezing operations are the greatest way to preserve carcass, meat and meat products for a longer time which inhibits bacterial growth, but not the psychrophiles and the spores. Most of these survive freezing and grow during thawing (Neumeyer *et al*., 1997).

Traditional methods for preservation of meat by salting and picking are well accepted procedures. Other chemicals have been used as food additives for preservation of meat but every country has drawn its rules and regulations and established limits for the purpose of prevention of harmful effects to human (Cassens, 1997). The meat industry over the years uses salts of nitrates and nitrite to prevent the growth of *Clostridium botulinum*, provide the products with a pinkish color, cured meat typical flavor, and possess antioxidant effects. The major dietary resource to nitrite and nitrosamine is associated with cancer (Liu *et al.*, 2009).

According to epidemiology and clinical studies, high intake of dietary nitrate and nitrite have been associated with etiology of human gastric cancers (Hsu *et al*., 2009).
Naturally nitrate are present in leafy vegetables while sodium or potassium salt of nitrite are added to meat as preservative (Hsu et al., 2009). In the oral cavity and stomach nitrates are converted to nitrites that react with amines and amides to form a group of carcinogens called N-nitroso compounds. The low pH in stomach promotes the formation of nitrosation (Hsu *et al.*, 2009).

The World Cancer Research Fund and American Institute of Cancer Research report, based on an extensive review of the existing evidence by an international panel of experts, concluded that a high intake of red or processed meats is a convincing and probable cause of colorectal cancer. The risk of colorectal cancer was estimated to increase by 29% for every 100 g/day increase in red meat and by 21% for every 50 g/day increase in processed meat consumption (Kim *et al.*, 2013).

However, these additives can cause serious damages to health; the nitrite combines with the secondary and tertiary amines present in the meat, creating nitrosamines that are highly carcinogenic substances. They can also combine with hemoglobin creating methemoglobin, which damages the blood oxygen transport. The long term nitrite intake can cause methemoglobinemia, mainly in children, and the nitrite intoxication causes vasodilatation, smooth muscle relaxation, face redness, gastrointestinal discomfort, headache, cyanosis and vomiting. This can be fatal, particularly in newborn infants in which the methemoglobinreducing capacity is low, leading to so-called `blue baby syndrome' (Vasco and Alvito, 2011).

The n-nitroso compounds formed from the nitrite reactions with the amines are mutagenic and teratogenic. The risk of colorectal and stomach cancer have suggested to increase with intake of red and processed (nitrite-preserved red) meat according to epidemiologic studies with processed meat showing higher risk estimates per gram of intake than red meat (Essumang *et al.*, 2012). .

Nutrition has been implicate in playing complex role in cancer etiology. Heterocyclic amines, known carcinogens are formed during cooking of meat at high temperatures, but this is not specific for red and processed meat (Essumang *et al.*, 2012). Nitroso compounds can be formed via nitrosation of organic compounds in meat by nitrite, one of the methods of preservation. Endogenous N-nitrosation of amines forms nitrosamines. Several N-nitroso compounds are known carcinogens and can alkylate DNA leazvding to G-to-A mutations. Heme iron, abundantly present in red meat, also has a catalytic effect on the endogenous formation of Nnitroso compounds (Kim *et al.*, 2013).

Estimates suggest 34,000 deaths from cancer every year could be due to diets high in processed meat (Citi 97.3 FM, 2015). It is estimated that 16,600 cases of cancer occur annually in Ghana with an occurrence rate of about 109.5 cases per100, 000 persons according to Cancer Control Division of Ghana Health Service (GHS) on February 4, 2011. The report stated that most of the cases seen in Ghana and other West African countries identified the disease with younger people, which is the direct opposite of what has been reported in the developed world (GNA, 2011).

Another statement by the CEO of the non-governmental organization Breast Care International on March 22, 2011, indicated that breast cancer cases among Ghanaian women are on the rise.

It is very possible that high levels of curing agents in Ghanaian diets from processed meat may be contributing to some of these increased cases of cancer, especially breast cancer among Ghanaian women (GNA, 2011).

Due to the growing concern of N-nitroso compounds, it is therefore necessary for long-term monitoring of nitrate and nitrite concentrations in foods for susceptible populations.

This study, therefore, seeks to determine the levels of residual nitrite in some processed meat in Ghana. The data from the study will also be used to assess health risk of consumption of nitrite preserved meat.

1.2 Problem Statement and Justification

The International Agency for Research on Cancer (IARC) of World Health Organization has evaluated the carcinogenicity of the consumption of processed meat and red meat (Bouvard, 2015). Processed meat is a delicacy in several parts of the world including Ghana. Its processing involves curing of the meat with nitrites and nitrates to prevent bacterial spoilage, enhance colour, flavor and texture of the product.

However, these additives can cause serious damage to health; nitrites combines with amines present in the meat creating nitrosamine that are highly carcinogenic substances. This present a significant health hazard to consumers and therefore the need to investigate the levels of nitrites in processed meat in order to assess the suitability for human consumption.

1.3 Objective of the Study

The main objective is to determine the residual levels of nitrites in some processed meat on the Ghanaian market and also assess the associated health risk of consumption of nitrite preserved meat.

The specific objectives are:

- To assess the residual levels of nitrites in processed meat
- To estimate dietary intake of Nitrite among processed meat consumers in the Ayawaso West Sub Metro, Accra.
- To estimate the health risk of consumers from exposure of nitrite.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat Role and Importance in Human Nutrition

Meat is a rich source of some importance micronutrient such as iron, selenium, vitamin A, B12 and folic acid which cannot be derived from plant food and if present have poor bioavailability. It is also rich protein source and low glycemic index which is beneficial respect to obesity and diabetes development etc. (Pereira *et al.*, 2013).

It is very important in human health and development because it is a reservoir of micronutrient and amino acid which are very essential in regulatory process of energy metabolism (Biesalski, 2005).

Red meats are abundant sources of iron, an element which is required by the body to build and maintain blood hemoglobin, which carries oxygen to body cells. Red meat is also a rich source of zinc, an essential trace element that contributes to tissue development, growth and wound healing (Abdul *et al.*, 2014).

2.1.1 The Role of Meat in Diet in Africa

In most communities meat has a prestigious value and are usually consumed during festive occasion and celebrations and regarded as food with higher nutritive value. In the developed countries most diets are typically dependent on cereals or root tuber crops with small amount of meat to complement the staple food (Bender, 1992). Meat, a rich source of iron improves iron absorption from other foods and its rich source of amino acids complement that of many plant foods. It is also sufficiently rich in vitamins especially the B-vitamins and very essential protein requirement for humans (Bender, 1992).

2.2 Preservation of meat

The major choice of animal protein for people all over the world is meat. It was reported that approximately 3.5 billion kg of meat and poultry go waste at the consumer, retailer and food service levels which has great deal of impact on the economy as well as the environment. Major cause of this lost is due to microbial spoilage (Dave and Ghaly, 2011). According to Cerveny *et al.* (2009) the 5% of the meat and poultry loss could satisfy the daily needs of approximately 320,000 people.

Production of meat from animals involve series of operational steps and this include transporting animals to slaughter houses , offloading and holding of animals, slaughtering of animals under poor operational techniques and facility which results in loss of meat, reduce meat quality and increase microbial proliferation. This therefore require the need to develop optimum preservation technique to maintain these products (Dave and Ghaly, 2011).

2.2.1 Meat Preservation Techniques

Animal flesh which serve as food is called meat. Due to the rich nutrient composition of meat, microorganisms and common pathogenic bacteria thrive in them causing spoilage and therefore very important to employ techniques that will maintain the safety and quality (Zhou *et al.*, 2010).

Meat preservation methods mainly inhibit microbial growth, however other methods reduce deteriorative changes such colour and oxidative changes. Factors influencing shelf life and quality of meat product include holding temperature, atmospheric oxygen (O2), endogenous enzymes, moisture (dehydration), light and, most importantly, micro-organisms (Zhou *et al* ., 2010).

The meat preservation methods commonly employed relies on control of moisture, temperature and microbial loads (through processes such as ionizing radiation, packaging etc.). Processed meats and poultry are extremely perishable products, and one of the principal agents of their spoilage is microorganisms (bacteria, yeasts, and molds). Microorganisms cause nutritional and sensory deterioration of meat products, producing loss of quality and limiting shelf life. Besides the economic implications of meat spoilage (deterioration of raw materials and processed products before they can be sold, loss of brand image, etc.), microorganisms can also be responsible for human illness (Jiménez-Colmenero and Solana, 2007; Zhou *et al*., 2010).

A variety of preservation procedures have been tried to limit the speed and extent of such processes and their consequences. Traditionally, much use has been made of physical, chemical, and microbial methods of preservation. Preserving processed meats by chemical means is based, among other possibilities, on the use of additives known as preservatives. Preservatives are chemical compounds that, when added to foods, inhibit, retard, or prevent the activity and growth of spoilage and pathogenic microorganisms. Their chief purposes are to extend the shelf life of foodstuffs by protecting them against deterioration caused by microorganisms, and to enhance their safety. (Jiménez-Colmenero and Solana, 2007).

To ensure the efficacy of preservative used, control and regulation is much pivoted because these additives can have adverse health effects. Due to consumer protection the use of preservatives is bind to strict legal regulation , which creates the awareness of aspects of food that affect health, most especially the presence of additives, and among these preservatives.

Legislative requirements relating to the use of preservatives in meat products are regulated by the European Union (EU) through various European Community Directives. (JiménezColmenero and Solana 2007).

2.2.2 Refrigeration

Meat produced under strict hygienic conditions can be stored for several days under refrigeration temperatures of 0 – 4^{o}C and when packaged under carbon dioxide or nitrogen or in vacuum, period can be extended to 10 days (Bender, 1992).

Commercially freezing at -29°C and domestically at -18°C, meat preserve best for a periods of 1-2 years but there is some deterioration of eating quality compared with fresh or chilled meat.

2.2.3 Canning

Canning has been adopted for several decades and plays a significant role within the economy in the food processing sector (Simpson *et al.*, 2003). Canning preserves food for many years due to protection from recontamination (Bender, 1992). As a result, it allows consumers to have varied nutritious diet at all seasons irrespective of geographical location.

2.2.4 Smoking

Smoking is one of the oldest preservative method for meat- traditionally over a wood fire and more recently by producing smoke from wood sawdust in a generator and conducting the smoke over the meat. In region of high air humidity there is the risk of mould growth on the surface of meat and the antimicrobial effect of smoke can prevent this occurrence. For instance, many of the larger raw-cured meat cuts are treated with application of short sequence cold smoke (around 20^{o}) to enhance preservation. The substances deposited on the meat contribute to the flavour and appearance but with ordinary, light smoking the preservative effect is limited and the product has to be stored refrigerated (Gunter and Hautzinger, 2007).

Intensive smoking does prolong shelf life both by heavier deposition of preservatives and by the drying effect of the hot air but it has a detrimental effect on flavour. Consequently preservation by smoking is regarded as an emergency measure when other methods cannot be used. A modern development making use of the flavouring effect is to use an aqueous solution of the constituents of smoke which reduces the amount of strongly flavoured and other unwanted substances (Gunter and Hautzinger, 2007).

2.2.5 Curing

Curing is preservation with salt (sodium chloride) and sodium or potassium nitrite or nitrate or a mixture of these two salts. The nitrate are decompose by bacteria during the curing process to form nitrite. The oldest method of meat preservation is salting. Salt concentrations greater than 4% in the aqueous phase inhibits the growth of most spoilage organisms. For complete preservative effect the salt concentration is usually around 17%, and this mostly make the product unpalatable. In most cured meat products the salt concentration is between 2.5 and 5% and the nitrite inhibits the growth of other organisms. Nitrite also reacts with proteins when heated to form compounds called Perigotype factors that inhibit the development of spores of *Clostridium botulinum*, the cause of botulism, the most serious type of food poisoning. Additionally, nitrite is broken down to nitric oxide, which reacts with the red colouring matter in muscle, myoglobin to form deep-red nitrosomyoglobin. As the protein is denatured, this is converted, rapidly when heated and more slowly otherwise, into a pink compound which is responsible for the typical colour of cooked ham, canned luncheon meat, franfurters and raw ham, dry sausage, etc. (Sullivan, 2013).

The early curing procedures were lengthy and recent developments have led to a reduction in the time required. For example instead of simply immersing the meat in brine it is first injected with the curing solution and the process can be completed in 1 - 2 weeks. Thin slices of meat such as bacon can be cured in a few hours, and the processing time can be reduced to a few minutes if heated and if the cure is completed in the final package (Sullivan, 2013).

Meat curing involves a highly complex series of reactions involving nitrite and a wide range of substrates that produce the end products responsible for cured meat properties. Nitrite is typically added as an ingredient during formulation of cured meats (Sullivan, 2013). However, recent development of natural and organic processed meats has required use of alternative curing processes because USDA regulations do not permit direct addition of nitrite to these products. One of the alternative processes that have been developed utilizes celery powder as a natural source of nitrate and a bacterial starter culture that provides the reduction of nitrate to nitrite to achieve curing reactions (Sullivan, 2013).

Because production of nitrite by bacterial action is slow, and nitrite reactions with meat components range from slow to fast, we hypothesized that slow production of nitrite in natural and organic products would favor a greater proportions of end products from substrates that react quickly with nitrite at the expense of those that are relatively slow to react. Changing the relative amounts of end products of curing could have implications for cured meat properties such as microbial control, a property that has been reported to be less effective in natural and organic products than in conventionally-cured meats. (Sullivan, 2013).
Animal experiments have shown potential risk from nitrosamines formed from nitrite. The addition of sodium ascorbate to the pickling brine accelerates the curing process because of its reducing capacity and allows smaller amounts of nitrite to be used, so there is less residual

nitrite in the meat which reduces the possibilities of the formation of nitrosamines. Residual ascorbic acid has an antioxidant effect in stabilising the colour and preventing rancidity.

2.2.6 Tumbling and Massaging

This is a technique which permits maximum penetration of salt into meat during curing. The process involve injecting pieces of meat with the curing salt solution or chopped meat immersed in it and mechanically shaken-" tumbled". Solutions of 2-8% salt are used, sometimes with the addition of polyphosphate, when there is some extraction of water-soluble protein, mainly myosin. The effect is to improve the water-holding capacity of the meat by reaction between the salt and the structural proteins, aided by the polyphosphate. The extracted proteins set to a strong gel on heating and so bind together the pieces of meat, which can then be shaped or sliced. The term "massaging" is applied to a relatively gentle mechanical treatment while "tumbling" is a more vigorous action (Bender, 1992).

2.3 Processed Meat

Meat that has been subjected to any process which alters its original state, excluding sectioning, and freezing, with or without other ingredients, and which as a result of this process or these processes the product is irreversibly changed. The properties of fresh meat are modified by the use of procedures such as mincing, grinding or chopping, salting and curing, addition of seasonings and other food materials, and, in many instances heat treated. The Processing usually involves the mixing of other foodstuff such as cereals with the less desirable part of carcasses and lean meat to extend meat supplies (Abu, 2016).

2.3.1 Meat Processing by Comminution

The breaking down of whole muscle meat into small particle size is referred to as comminution.

There are various comminution levels among processed meat product that is finely, coarsely and to form an emulsion. Meat trimmings, meat pieces and fatty tissues that could otherwise not be utilized are comminuted and spiced to form high quality meat products. Typical examples of comminuted meat products include; sausages, meat loaves, burgers and liver patties (Abu, 2016).

2.3.2 Processed (Common Cured) meat products in Ghana

Globally, the intake of meat products is high and still escalating particularly in developing countries (Cawthorn et al., 2013). Ghana is ranked the highest consumer of meat products in Africa followed closely by South Africa (Cawthorn et al., 2013). In Ghana, the commonest meat products include: sausages, bacon, ham, corned beef, hot dogs, salamin etc.

2.3.2.1 Sausages

Comminuted meat seasoned with spices and stuffed into casings are known as Sausages. They may be cured, dried or fermented. They are made from any edible part of animals and some non- meat ingredients (Heinz and Hautzinger, 2007). Meat processing provides farmers with a ready market for their animals thereby providing necessary encouragement for improved and increased production (Abu, 2016).

There are about 800 varieties of sausages but six main types available are fresh, smoked, cooked, smoked and cooked, semi dry and dry worldwide (Heinz and Hautzinger, 2007).

Various kinds of meat are used in the manufacture of sausages e.g. beef, pork, mutton, chicken etc. (Abu, 2016). The chopped meat are seasoned with salt and spices mixed with cereal and packed into natural casing i.e. tissue of animal intestines or cellulose, collagen or synthetic material. Examples of sausages include fermented sausage such as salami, liver sausage, blood sausage, and pepperoni, frankfurters etc. (Abu, 2016).

2.3.2.2 Bacon

Bacon is cured pork made from specific parts of the pig and sometimes any part of the animal by dry curing or in pickles sodium nitrite and smoked. Bacon varied according to the curing process and the amount of fat. Late in the 19^{th} century bacon became an important international trade commodity due to its long storage shelf -life. Bacon is widely enjoyed for its unique, smoky flavour. It is a favourite with eggs and is used in the preparation or garnishment of a wide variety of dishes such as pizza (Ibarra, 1990).

2.3.2.3 Ham

Ham is the cured product of the upper leg and buttock of the pig. It is stable when raw after a certain period of maturation but is often cooked to pasteurisation temperature, 70°C, or it may be canned at pasteurising temperature. It may be smoked as an additional means of preservation and flavouring. It is mostly enjoyed as garnish in varieties of dishes (Ibarra, 1990)

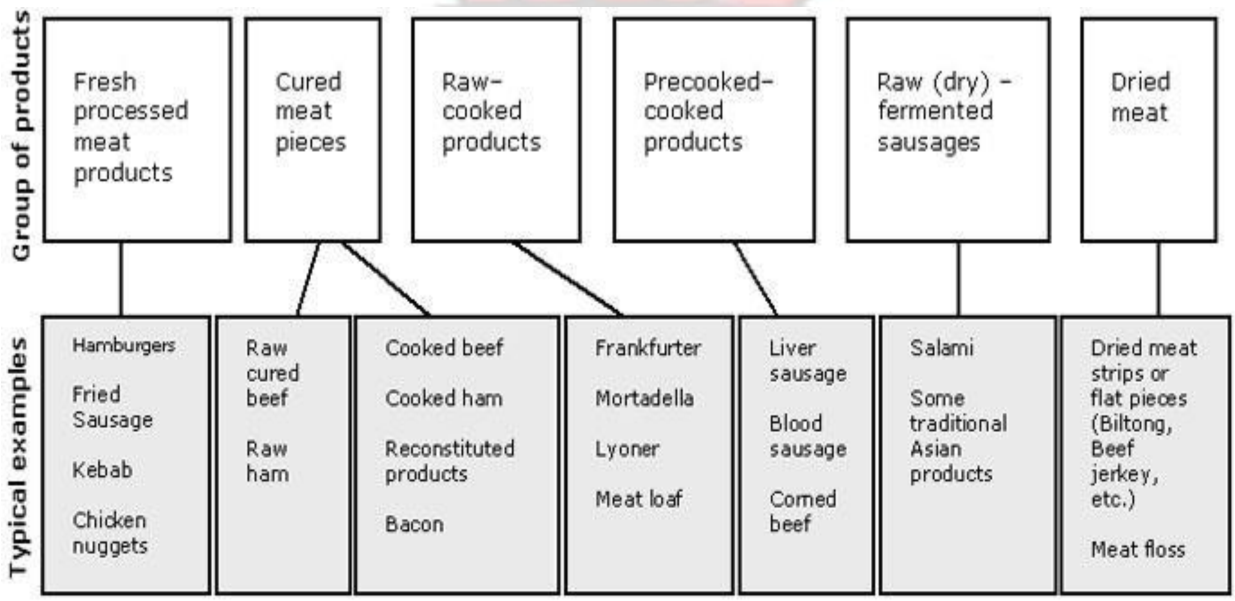
2.3.2.4 Corned Beef

These are usually produced by hot water extraction from meat of poorly pastured animals with retarded growth but rich in connective tissues as well as muscle. The exhausted meat from extraction are cured with coarse grains of salt (“corn”) and nitrite and canned. Other meats such as mutton can be treated similarly and some modern processes use extracted beef (Ibarra, 1990).

2.3.2.5 Burger

Patties made from minced meat have become popular world-wide through the agency of international fast-food outlets. These were originally hamburgers made from beef with the name derived from the Hamburger sausage but may be made from any meat and "mutton burgers" are marketed in regions where beef is not eaten. The meat content varies from 100% including about 20% fat, to 80% or less with various additions of cereals, onion and water. Since the pattie is raw it is stored frozen (Ibarra, 1990)

Figure 2.1 Meat products grouped according to different processing technology



2.4 Properties of Nitrites

Sodium nitrite is the inorganic compound with the chemical formula $NaNO_2$. It is a white to slightly yellowish crystalline powder that is very soluble in water and is hygroscopic. It is a useful precursor to a variety of organic compounds, such as pharmaceuticals, dyes, and pesticides, but it is probably best known as a food additive to prevent botulism (National Center for Biotechnology Information, 2005)

2.5 Chemistry of Nitrate and Nitrites

Nitrates and nitrites are salt of sodium or potassium that has been used for thousands of years to preserve meat well before the discovery of refrigeration. The use of nitrite and nitrate salt in meat processing enhances the red meat colour, inhibit some spoilage and food poisoning anaerobic microorganisms, delay the development of oxidative rancidity, and contribute to flavor development.

About 2% of nitrite is usually used in the meat curing process and this generates approximately 150ppm of nitrite content in the product. This amount is not toxic to consumers, however upon reaction with myoglobin in meat an average residual between 50-100ppm remained (Heinz and Hautzinger, 2007).

The maximum ingoing amount of nitrite for processed meat products is normally up to 200mg/kg of product and the residual nitrite in a finished product should not exceed 125ppm (Session, 1998).

Due to the type of processing method and condition as well as other factors the nitrite react with many component in the matrix (myoglobin, non heme proteins, and lipids) such that the nitrite detected does not reflect the initial amount added. Nitrite levels in meat products are important because nitrite can react with secondary amines to form nitrosamines, which are recognized as having carcinogenic effects (FAO/WHO (2003a).

In the EU, potassium and sodium nitrite and nitrate are authorized for use in different meat products, and maximum ingoing amounts (150 mg/kg for nitrite and 300 mg/kg for nitrate) are established for all products, as well as maximum residual levels for some of them. Current regulations on use of nitrite and nitrate vary depending on the curing method used and the product that is cured. For comminuted products, the maximum ingoing nitrite and nitrate limits are 156 mg/kg and 1718 mg/kg, respectively. For immersion-cured and massaged or pumped products, those limits are 200 mg/kg and 700 mg/kg, respectively. In dry-cured products nitrite is limited to 625 ppm and nitrate to 2187 ppm. The ADI for nitrites, as nitrite ion, is 0.07 mg/kg body weight, and 3.7 mg/kg for nitrate, as nitrate ion (FAO/WHO (2003a),

To detect the presence of nitrite ion in food three methods are commonly used namely chromatography, spectrophotometry, potentiometry, and use of microbial biosensors. But the most sensitive method is the spectrophotometric determination of nitrite. The reaction involves conversion of nitrite to nitrous acid in acidic medium followed by diazotization of sulphanilamide or sulphanilic acid and formation of diazonium salt. The diazonium salt was then coupled to either N-(1- naphthyl ethylenediamine or 1-naphthylamine to form azodye measurable at 540 nm (Hassan *et al.*, 2003).

2.5.1 Ascorbates and Erythorbates

When nitrite is used in curing meat**,** Ascorbates and erythorbates are used interchangeably to enhance the curing process. These chemicals are active reducing agents that react with nitrite to accelerate the curing process. Ascorbate is derived from ascorbic acid (Vitamin C) (Heinz and Hautzinger, 2007).

2.6 Dietary Sources of Nitrates and Nitrites

Diet remain the main source of nitrate and nitrites to human and these are mainly obtained from Plant foods such as leafy vegetables, water and cured processed meat products (Thomson *et al.*, 2007).

2.7 Absorption and Distribution of Nitrate and Nitrite in the Body

Nitrate and nitrite can be inhaled from the environment into the body but the amount might not cause any adverse health effects. However, major route of nitrate and nitrite are through the ingesting of water, food and soil that contain these substances. Intake of some nitrate is a normal part of the nitrogen cycle in humans. Both nitrate and nitrite can be produced inside the body as well. Some of the nitrate in your body moves from blood to the salivary glands where some of it is changed to nitrite. Nitrate and nitrite are widely distributed in the body. Nitrate and nitrite that enter your body are no different chemically than nitrate and nitrite produced inside your body. Most nitrate in your body leaves in the urine the same day it enters the body. Some nitrite in the stomach forms other substances, some of which may be harmful. Nitrite in the blood can react with hemoglobin (which carries oxygen to body tissues) and reduce the ability of hemoglobin to carry oxygen. Nitrite can also form nitric oxide, which may be beneficial in some instances (Division of Toxicology and Human Health Sciences- nitrate and nitrite, 2015).

2.8 Harmful Effect of Nitrates and Nitrites

When nitrates are converted to nitrite and nitrosamine it become toxic to human. According to literature the lethal oral doses of nitrite and nitrate for human are established in 80–800 and 33–250 mg/kg body weight, respectively (Larsson *et al.*, 2011).

It has been established that nitrites are formed during reduction of nitrate by bacteria in the saliva and under gastric acid condition in reaction with secondary amine to form the carcinogenic nitrosamine. Consequently, it estimated that nitrite is 10 times more toxic than nitrate and it's potential as a human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC). In adults, 5% to 7% of all nitrates that enters into the body is converted to nitrite. The amount of nitrite conversion in young people and persons with gastrointestinal illnesses is greater due to lower stomach pH. Due to the oxidative and reductive status of the gastrointestinal tract the presence of nitrate and nitrites in food could be considered hazardous. The compounds produce as a result of these reaction harmful effect on the human health. The compounds known as N-nitroso compounds are easily formed by the interaction of a secondary amino compound with nitrite and nitrate in foods. N - nitroso compounds have been revealed to induce cancer in many tissues such as lung, kidney, liver, bladder, pancreas, esophagus and tongue, brain, colon and bone, depending on the species (Larsson *et al.*, 2011).

In the presence of acid or heat from cooking, nitrosamine is converted into a di-azonium electrophile and similar ions which reacts with biological nucleophile molecules, such as DNA and proteins. The substitution reactions of biomolecules can disrupt normal cell function and lead to cancer or cell death. Animal studies in various species have provided reliable evidence for carcinogenesis in multiple tissues, such as the colon, bronchus, stomach, and pancreas, due to nitrosamine compounds (Larsson *et al* 2011).

The teratogenic effect of nitrate has caused an acceptable daily intake (ADI) to be determined for nitrate and nitrite (Larsson *et al.*, 2011).

The most important human and livestock disease caused by the presence of nitrite in food or water is methemoglobinemia, or blue baby syndrome. In this disease, nitrate in the digestion system leads to the production of nitrite. The high aqueous nitrite concentration allows it to pass easily from the digestive tract into the blood. In the blood, nitrite oxidizes the iron in hemoglobin, converting it to iron which creates methemoglobin, a brown pigment unable to transport oxygen (Larsson *et al.*, 2011).

This process disrupts oxygen flow to the body's tissues, and in the absence of oxygen, the cells of the body become disabled, turning the skin, lips, and nails gray. Other symptoms of this illness include diarrhea, vomiting, lethargy, and malaise, and the last stage of the disease leads to suffocation and death (Larsson *et al.*, 2011).

The methemoglobin reductase enzyme is produced in adults, which converts this compound to oxyhemoglobin. Due to a lack of this enzyme, however, several groups of people are still vulnerable to methemoglobinemia, including children, pregnant women, gastritis patients, and people with a congenital deficiency for this enzyme. As a result, nitrate and nitrite in food has been associated with increased risk of stomach cancer and intestinal disease in adults, and methemoglobinemia in infants (Larsson *et al.*, 2011).

The methemoglobin level in the body is usually between 1% and 3%. When the concentration of methemoglobin reaches more than 1.5 g/dl, or more than 10% of an adult's hemoglobin concentration, it becomes important clinically (Larsson *et al.*, 2011).

2.8.1 Toxicity of Nitrate and Nitrite

Nitrite has been implicated in a variety of long term health effects and the toxicity of nitrite has been evaluated several times as new information has been published. The most recent evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is based on the effects on the heart and lung in a 2-year rat study. This study is the basis of the current

Acceptable Daily Intake (ADI) of 0-0.07 mg/kg bw/day (as nitrite ion). Nitrite may also combine with secondary or tertiary amines to form N-nitroso derivatives. Certain N-nitroso compounds have been shown to produce cancers in a wide range of Laboratory animals (Thomson, 2004).

It has been established that fatal dose of nitrate for adult humans is in the range of 30 to 35g consumed as a single dose whiles for nitrite in a range of 22 to 23g per kilogram body weight. The lower dose particularly causes methemoglobinemia in children. It has been estimated that 10 percent of the human exposure to nitrite in the digestive tract comes from cured meats and 90 percent comes from vegetables and other sources. For an adult of approximately 70kg to be expose to 22mg of sodium nitrite per kilogram body weight (lethal dose) would have to consume about 8.5kg of cured meat product containing 200ppm. According to a report by National Institute of Occupational Safety and Health of USDA there are data indicating nitrite is a primary irritant, tumorigen, mutagen and that it causes reproductive effects (Chamandoost *et al***.,** 2016).

Other health effects following fetal exposure to elevated levels of nitrates in drinking water included intrauterine growth retardation, increased incidence of Sudden Infant Death Syndrome (SIDS), cardiac defects, and increased risk of nervous system defects. The U.S. EPA concluded that there was conflicting evidence in the literature as to whether exposures to nitrate or nitrites are associated with cancer in adults and in children. The types of cancers studied included non-Hodgkin's lymphoma as well as stomach and gastric cancers in adults; and brain tumors, leukemia, and nasopharyngeal cancers in children. Maternal (during pregnancy) or child consumption of nitrite-containing meats may be associated with increased incidence of brain tumors in children (Borchert, *et al.*, 1998).

A few studies have reported other health effects that are possibly associated with nitrate exposure in children, including increased incidence of childhood diabetes, recurrent diarrhea, and recurrent respiratory tract infections. Other reported effects of chronic exposure reported in adults include frequent urination and spleen hemorrhaging (bleeding). Acute high dose ingestion exposure to nitrates can cause abdominal pain, muscle weakness, blood in stools and urine, fainting, and death (Borchert *et al., 1998).*

2.8.2 Mechanism to Reduce the Use of Nitrite

Over usage of nitrite can be minimize when a mixture of common salt (Nacl) and nitrite are used. To achieve good results for the curing process a proportion of 0.5% nitrite and 99.5% sodium chloride is desirable (Honikel, 2008). Rock salts that naturally contain low levels of nitrites have been known for centuries to be excellent preservative agents. The reduction of lipid oxidation in meat and meat products by excluding oxygen and addition of ingredients with antioxidant potentials such vitamin E, lycopene or lutein can minimize the use of nitrite (Weiss *et al*., 2010)

2.8.3 Nitrosamines

Nitrosamines are produced when nitrite and nitrogen oxides react with secondary amines and N-alkylamides. They are usually present in processed cured meat products and can also be formed endogenously after consumption of red and processed meat (Ferguson, 2010).

Under condition not yet understood natural breakdown product of protein to amines react with nitrite to form nitrosamines. Not all cured meat products contain nitrosamines; when present, they usually are in very minute amounts. However, nitrosamines can only be found in strongly cooked or fried meat products which were previously cured with nitrite. Factors that affect nitrosamine levels include: amount of nitrite added during processing, concentrations of amines in meat, type and amounts of other ingredients used in processing, actual processing conditions, length of storage, storage temperatures, and method of cooking, and degree of doneness (Campillo *et al.*, 2011).

It is unknown at what levels, if any, nitrosamines are formed in humans after they eat cured meat products, or what constitutes a dangerous level in meat or in humans. Nitrosamines are found, very infrequently in all cured products. The use of nitrosating agents for curing meat is strictly controlled in some countries. However, some known inhibitors of the nitrosation reaction, such as ascorbic acid and tocopherol, are used in the processing of several foods. Food irradiation at the appropriate dose has proved to be a safe way of achieving safety in this respect by reducing the nitrosamine contents. The meat industry must be monitored to provide safe meat and to preserve human health (Epley., *et al* 1992).

2.8.4 Analytical Methods

Several methods have been reported for quantitative determination of nitrate and nitrite in foods, including spectrophotometry, chromatography and electrochemical detection (potentiometry, amperometry, polarography methods. However, not all of them are equally suitable for use on highly heterogeneous solid matrices like processed meats and poultry (Cammack, 1999)

The nitrite is extracted by dispersion in hot water and since meat products contain various compounds that can interfere in nitrite measurement, clean-up of the extracts is done prior to determination. These include clarification stages (fat and protein precipitation, filtration, etc.) using different compounds or solvents (Carrez or borax reagents, zinc sulfate or potassium ferrocyanide, acetonitrile, and others) or pretreatment cartridges to remove sample matrix interferences. Another possibility is to separate the fat by centrifugation and rapid cooling followed by in-line dialysis to remove protein and remaining fat. Obviously the choice of a specific procedure to clean up an extract prior to determination will depend on the analytical method used (Cammack, 1999)

2.8.5 Other Uses of Nitrite

Although nitrite is a controversial food additive, recent studies indicate that nitrite can inhibit the production of malonaldehyde, which may be toxic to living cells. In small quantities. malonaldehyde frequently is found in food products that turn rancid. Wieners, ham, bacon, and corned beef resist the accumulation of malonaldehyde due to their nitrite content (Lundberg and Weitzberg, 2005).

Most of the industrial uses of sodium nitrite are based on its oxidizing properties or its liberation of nitrous acid in acidic solutions. Sodium nitrite is a convenient source of nitrous acid in the nitrosation and diazotation of aromatic amines and the production of azo dyes (Pokorny *et al.*, 2006).

Other applications of sodium nitrite include the syntheses of saccharin, synthetic caffeine, fluoroaromatics and other pharmaceutical products (Butler and Feelisch, 2008).

Pesticides and organic substances; as an inhibitor of polymerization; in the production of foam blowing agents; in the removal of hydrogen sulfide from natural gas; in textile dyeing; and as an analytical reagent (Pokorny *et al.*, 2006).

Sodium nitrite can be used as an antidote for cyanide poisoning and as a vasodilator (Lundberg and Weitzberg, 2005).

2.9 Risk Assessment

The evaluation of the risk and harmful health effect of chemical substances to humans is termed risk assessment and it involves four fundamental basic steps namely hazard identification, dose response assessment, exposure assessment, and risk characterization.

2.9.1 Hazard Identification

This process deals with identifying a potential adverse effect in human associated with exposure to a chemical, the possibility of such effects occurring and certainty or uncertainty associated with such effects and the circumstances under which the hazard may be expressed (FAO/WHO, 1995).

Conducting hazard identification require weight of evidence approach which in effect utilize review of all scientific information from epidemiological studies, animal toxicological studies, in vitro assays and quantitative structure-activity relationships (FAO/WHO, 1995).

2.9.2 Dose Response Assessment

When a dose of a chemical is administered to an animal in a toxicological studies, at a given an adverse health effect is observed likewise when human ingest a food chemical to a certain threshold it elicit an ill health effect or response. Risk characterization is therefore very important in food safety assessment because it establish the Dose response relationship. Exposure to nitrites generally results in adverse health effect, i.e. there exist a threshold concentration where effect is observed. In such situations, establishing health-based guidance values such as ADI or RfD for the residual nitrite must be a key outcome of the risk characterization process (FAO/WHO, 2009).

2.9.3 Exposure Assessment

In this assessment a real life situation which takes into accounts identification of potentially exposed populations; identification of potential exposure pathways; and quantification of the magnitude, frequency, duration and time-pattern of contact with a chemical (potential doses) (Yarmohammadi et al., 2015).

Several scenarios can be used to estimate exposure to a hazard. One method uses food consumption surveys in combination with levels of the chemical of interest in the food items. This method provides deterministic or probabilistic assessments. Food consumption can be derived from individual data or from per-capita estimates; the latter are the only available and comparable data at international levels (FAO-WHO, 2003a).

Levels of nitrates and nitrites can be determined in raw commodities or in food as consumed, in 'total diet studies'. Levels in food as consumed are closer to real intake than those in raw commodities (WHO, 2006b). Another method is based on the analysis of the total content of a meal or of a diet during one or several days of a limited number of subjects, and is known as the 'duplicate diet method'.

2.9.4 Risk Characterization

Risk characterization combines the results from the hazard characterization and the exposure assessment to estimate the potential risk to human health under different exposure scenarios including any assumptions. In this study, the toxicity was classified as non-carcinogen. The potential risk is compared between chronic daily intake (CDI) and reference dose (RFD) in hazard quotient (HQ) formula for residual nitrite. For multiple substance or multiple routes, the hazard index (HI) is used to calculate the non-carcinogenic risk. If calculated HQ > 1, there is concern that there may be excess non-carcinogenic Risk (Kaewrueng et al., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

The processed meat samples (sausage, corned beef, bacon) were obtained from supermarket, mini market , open markets and cold stores in Ayawaso West Sub Metro in Greater Accra Metropolis and transported in a cold chain stored in plastic bags and then kept in a refrigerator prior to analysis. Sulphanilamide, N-1-Naphthylethylene diamine dihydrochloride and sodium nitrite used for the analyses were purchased from BDH Chemical Ltd, England.

3.2 Methods

3.2.1 Study Population

The study was conducted in the capital city, Accra. This is because meat is a highly-priced commodity and therefore not likely to be consumed by population with financial pressure (Cawthorn et al., 2013). Specifically, the Ayawaso West Sub Metro was selected for this study because of its dense population with diverse social class relative to other Sub Metros (GSS, 2014). A total of 300 people of both gender were randomly recruited. This study was conducted between June-October, 2016.

3.2.2 Study Sampling and Participation

After seeking the consent of the participant, those who were spontaneously interested in participating in the study were made to fill a self-administered questionnaire designed to obtain socio-demographic information as well as food consumption data.

3.2.3 Dietary and Lifestyle Questionnaires

Participants were made to complete a 24 h self-administered food frequency questionnaire. (As shown in Appendix 5). First, the content of the questionnaire was explained to the participants. The questionnaire required that, participants describe the types and amounts of selected processed meat and suspected to contain nitrite which is usually seen to be commonly consumed in Accra. The list of processed meat in the questionnaire for participants to choose from were; *sausages, salami, hotdogs, bacon, ham and corned beef.* The participants were made to give information on specific meat among the list provided in the questionnaire which they have consumed within 24 h and over period of time from the time the questionnaire was administered to them. They were also supposed to provide the number of portions of specific processed meat they choose as well as the number of times they consumed per day. Real food models of standardized portions were used to help participants to better describe amounts weight. The questionnaire also required participants to provide socio-demographic data on level of education, body weight and height and their ages. The actual weights and heights of participants were measured. The questionnaire was pre-tested before the study.

3.2.4 Selection of Most Frequently Consumed Processed Meat Based On Data Provided By the Participant

The database of the data obtained from the 300 participants were imported into Epi-info 7 (2013) and the food consumption was analyzed using frequencies and percentages. The food with the highest percentages was taken to be the most consumed processed meat by respondents.

Sampling was done for the meat identified to be the most often consumed by the participants, on the basis of response to the questionnaires. Since levels of nitrite can vary considerably in the same product, the food sampling plan took into consideration both the types of consumed processed meat and variability within a batch and between batches. Thus, a total of 50 samples (namely 15- Chicken Sausages, 3-Pork Sausages, 15-Beef Sausages, 12-Corned Beef and 5Bacon) of commonly consumed processed meat based on availability were randomly purchased at supermarkets, minimarkets, and cold stores in the Ayawaso West Sub Metro, Accra. The samples were coded 1-50. Five grams of each coded sample was transferred into 50 ml beaker and 40 ml of water heated to 80^0C added. It was mixed thoroughly with glass rod taking care to break all lumps and transferred to 500 ml volumetric flask. The beaker and glass rod were thoroughly washed with successive portions of hot water adding all washings to the flask. Enough hot water was added to bring volume to about 300ml and transfered to steam bath and allowed to stand for 2 h with shaking occasionally. Cooled to room temperature, it was then diluted to volume with water and remix and filtered.

3.2.6 Determination of Nitrite

Standard calibration curve was used to determine the concentration of nitrite in the samples. Standard solutions of 0 .01, 0.02, 0.03 and 0.04 mg/l of $NaNO_2$ was prepared from a stock solution of 1.0 mg/L and measured using Cole Parmer UV-Vis (Model UV- 2150). The spectrophotometer was ―zeroed using the blank before standards and samples were measured. A calibration curve was established by plotting absorbance against the corresponding concentration. Using the equation of the calibration curve, the nitrite in test samples were determined. Determinations were done in triplicates.

3.2.7 Risk Assessment for Chemical Hazards

The chemical hazard concentrations in the processed meat products were determined and labelled as hazard content. It then used in models stated in the statistical design of the study to assess the risk. The USEPA guideline was use to evaluate the adverse health effect of hazard content determined by the nitrite levels in the various meat product. A uniform distribution fit was ran to determine a single concentration of all the nitrite levels in the meat product and that was used as hazard content in assessing risk of nitrite (Yarmohammadi, *et al.,* 2015). The Table 1 (Appendix 3) shows nitrite levels of processed meat product of the experimental samples.

3.2.8 Dietary Nitrite Intake and Risk Assessment

Modal food was obtained from data collected based on the 24 h food frequency questionnaire using Epi-info 7 (2013) designed by the Center for Disease Control (CDC), USA.

3.3 Statistical Analysis

The questionnaires filled by subjects and the gathered data about nitrite residual and amount of consumed meat products by the study population went under statistical analysis by statistical software SPSS version 16 at a significance level of 5%. ANOVA test, Tukey HSD option were applied. In order to calculate the health risk of a populace that are exposed to a chemical hazard, the probability of getting the endpoint disease (not the probability of dying of the endpoint disease) and the associated dose, consist of an average taken over an assumed 70-year human lifetime if dose is carcinogenic and 30 years if non carcinogenic. In order to correct all uncertainties, exposure to nitrites were calculated considering 10,000 iterations using the Monte Carlo simulation technique which is based on @RISK software (Palisade, US-version

6) carried out using Microsoft Office Excel 2013.

In calculating the risk, distribution characteristics of each exposure parameter were tested according to the exposure results. This eventually helps in determining the probabilistic distribution of the exposure dose after simulating result. The use of Monte Carlo simulations in risk assessment provides an understanding of the degree of uncertainty and variability around a risk estimate that single-point estimates of risk cannot provide (EPA, 1992). The mean exposure concentration of contaminants was used with exposed population variables and the assessment determined variables to estimate contaminant intake.

Chemical hazard intake is calculated using:

$$CDI = \frac{C \times CR \times EFD}{BW} + \frac{1}{AT} \qquad (1)$$

Where:

CDI is Chronic daily intake; the amount of chemical at the exchange boundary (mg/kg day). C is Average exposure concentration over the period. CR is Contact rate, the amount of contaminated medium contacted per unit time. BW is Average body mass over the exposure period (kg). AT is averaging time; the period over which the exposure is averaged (days). EFD is Exposure frequency and duration, where it is divided into EF is exposure frequency (days/year) and ED is exposure duration (years).

Table 3.1 Shows Statistical Distribution of Dataset Used In the Computation of CDI for Nitrite in the Ayawaso West Sub Metro-Accra

Variables	Statistical Distributions
Nitrite ingestion	Risktriang (15.46,15.46,388.63)
Contact rate	RiskGeomet (0.0068966)

Exposure Frequency	RiskIntUniform (52, 312)
Body weight	RiskNegbin (19, 0.25703)

After the CDI is obtained, a comparison between the CDI and the RfD should be done, if the CDI is below the RfD, it is assumed that the risk is negligible for almost all members of an exposed population. If it is greater the difference between the CDI and RfD is multiplied with the PF to obtained the risk of the populace to the endpoint disease of the hazard.

The general equation for calculating risk of chemical toxicant is:

$$Risk = PF(CDI - R_f D) \tag{2}$$

However in the absence of the reference dose of toxins (thus if a little of such reference toxins availability is assumed to have a risk, then there will be no level of which it is assume as safe for consumption, therefore $R_fD = 0$).

$$Risk = PF \times CDI \tag{3}$$

If the cancer oral slope factor or PF is not known, the hazard quotient (HQ) is calculated as risk.

$$HQ = \frac{CDI}{R_f D} \tag{4}$$

It has been established that a HQ value more than 1 (HQ >1) shows a significant risk level, While a HQ <1 shows no health risk on consumers (Moazeni *et al.*, 2014).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Demographic Characteristics of Study Participants

A total of 300 questionnaires were distributed but only 260 was analysed. One hundred and fifty females (57.7%) and 110 males (42.3%) were enrolled in the study. The mean age was 20.39 years with a minimum of 8 years to a maximum of 74years. The mean height and weight of the respondents were also 1.673m and 60.48kg respectively. Two hundred and ten of the respondents representing 81.1% had had tertiary education and were the majority at the time of the study. Table 4.1 is shows the demographic characteristics of the respondents.

Table 4.1: Socio-Demographic Characteristic of Respondents

Demographic characteristics	**Female n (%)**	**Male n (%)**	**Total n (%)**
Sex	150 (57.7)	110(42.3)	260 (100.0)
Age (years)			
Mean age ± SD	25±2.3	22.8±2.4	20.39 ± 1.6
Education level			
No formal education	6(2.3)	8(3.1)	14 (5.4)
Primary/JSS	30(11.5)	10(3.8)	40(15.4)
SHS	85(32.7)	35(13.5)	120(46.2)
Tertiary	50(19.2)	30(11.5)	80(30.8)
Religion			
Islamic	4 (1.5)	6(2.3)	10 (3.8)
Christianity	138 (53.1)	110 (42.3)	248 (95.4)
None	1 (0.38)	1(0.38)	2 (0.77)

4.2 Consumption of Processed Meat

The analysis showed that the most reported processed meat frequently consumed by the respondents were corned beef products, sausages and bacon. These accounted for 50.7%, 24.8%, and 24.5% for the rate of consumption of corned beef, sausage and bacon products three times per week respectively. The least reported consumed products were salami, hot dogs and ham corresponding to 75%, 70.9% and 64.5% of the respondents respectively. Figure 4.1 illustrates the distribution of consumption rate of the processed meat by the studied participants.

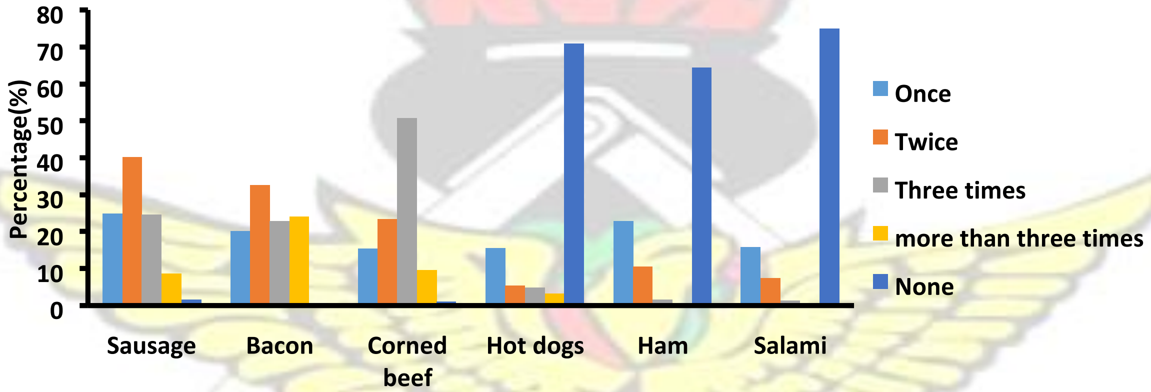


Figure 4.1: Distribution of consumption of processed meat of respondents

4.3 Statistical Results

4.3.1 Nitrite Levels in the Processed Meat Products

At a significance level of 0.05, the mean nitrite content in the processed meat is 139.85 mg/kg. We can conclude that processed meat consumed by the population in the Ayawaso West Sub Metro contains an average nitrite content of 139.85mg/kg. From Appendix 6, it can be observed that at most times processed meat from the study area has; minimum nitrite content (5th percentile) of 24.91 mg/kg and maximum nitrite content (95th percentile) of 305.14mg /kg). The median nitrite content was 124.74mg/kg and a modal nitrite content of 18.27mg/kg. The high levels of nitrite in the processed meat did not conform to the results from most international studies. A study in Denmark (Leth *et al.*, 2006) most processed meat products investigated did not exceeded the maximum permitted added amounts of sodium nitrite in Denmark (60 mg/kg for most products, up to 150 mg/kg for special products), except for a few samples which recorded higher values as 118mg/kg but did not also exceed the maximum residual limit stated by Danish veterinary and food Administration as well as WHO/EU rules. Another study conducted by Kamkar et *al* (2004) in Iran confirmed higher level of nitrite in their study which indicated high amount of residual nitrite (above permissible levlel of 60ppm by Iran) of salami and sausages tested. However, a study conducted by Tyrpenou et al. (2000) in Greece where 30 samples of pastirma (a traditional cured meat product) were examined recorded concentrations ranging from 0.85 to 189.65 mg kg-1 for nitrites, which is close to the results recorded in the current study. The variation between the residual nitrite content of the processed meat consumed in the current study and that of results from different studies is due to elapsed time of maintenance period, the pH of the meat, storage conditions and the different permitted levels of nitrite for processed meat products in different regions of the world.

4.3.1.1 Nitrite Levels in Bacon Brands

The mean nitrite levels in the bacon products ranged from a minimum of 38.4± 6.8 in back bacon to a maximum of 49.12±2.8 in collar bacon (Figure 4.1). Analysis of variance (ANOVA) at 95% confidence level revealed that there was a statistically significant differences in nitrite concentrations among the various bacon products ($p < 0.05$). However, when multiple comparism was conducted in means using Tukey's Honestly Significant Difference (HSD), it revealed that there was statistically significant differences in back bacon and collar bacon ($p = 0.032$), collar bacon and bitso bacon ($p = 0.044$).There were however no statistically significant differences among the following bacon products; Back bacon, hind bacon and streaky bacon

(Table 3.1)(Appendix 3). The nitrite levels recorded showed that they were within the standard range set by Ghana Standard Authority or FAO/WHO (125mg/kg).

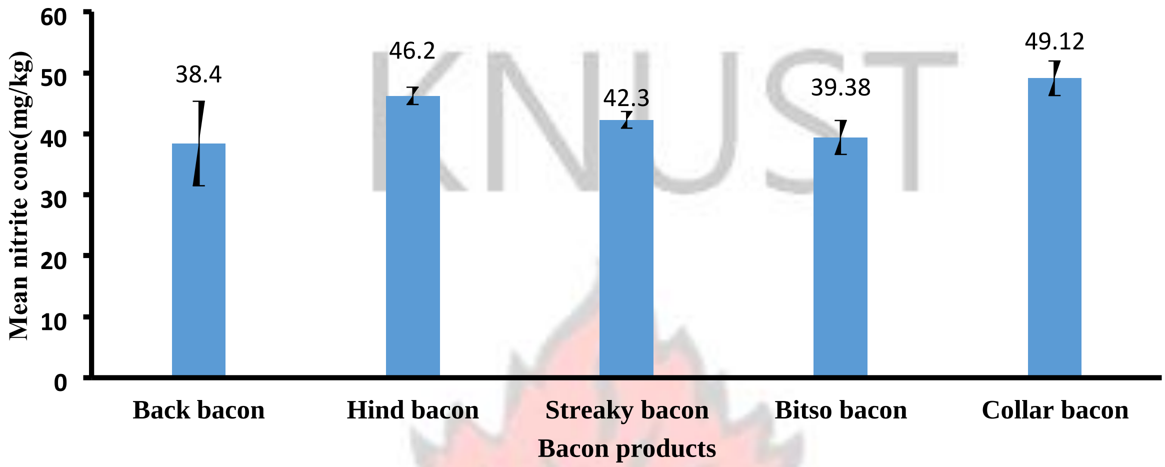


Figure 4.2: Mean nitrite levels of bacon products

4.3.1.2 Nitrite Levels in Beef Sausage Brands

The mean nitrite levels in the beef sausage brands ranged from 62.8± 19.27 in meaty beef to 343.5 ±11.02 in beef smokie (Figure 4.2). Analysis of variance (ANOVA) at 95% confidence level showed that nitrite concentrations among the various beef sausage brands differed significantly ($p < 0.05$). However, Tuckey's HSD multiple comparism revealed that there was no statistically significant differences between emborg beef brand and the following brands (beef khebab and bino) ($p > 0.05$). There were also no statistically significant differences among beef khebab, nempa sausage, dannka, adom, bino and beef smokie ($p < 0.05$) but were significantly different from the all other beef sausage brands ($p < 0.05$). The level of nitrite in all the beef sausages analyzed showed levels above the set standard with the exception of first sausage, meaty beef and Adom sausage. The entire analysis is shown in Appendix 3 (Table 3.2).

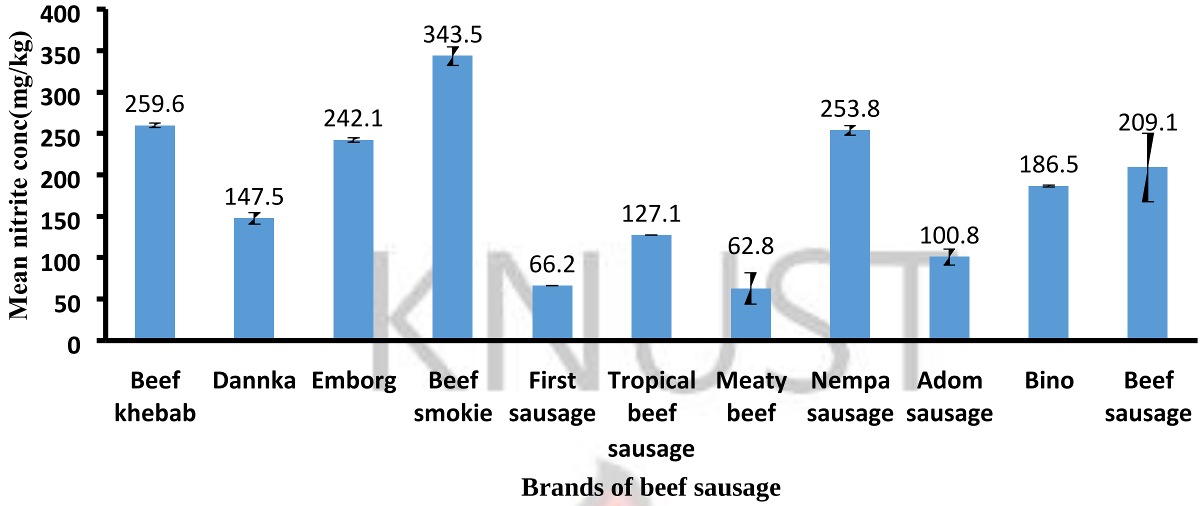
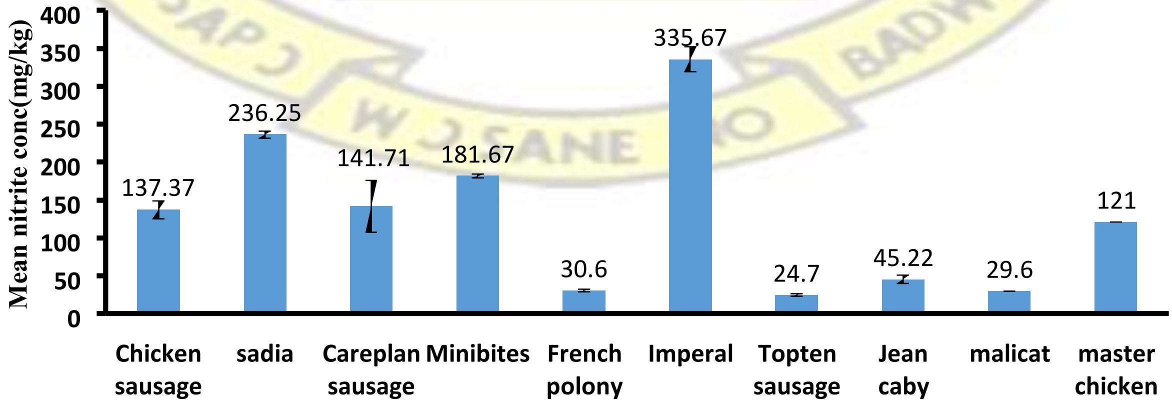


Figure 4.3: Mean nitrite levels of beef sausage brands

4.3.1.3 Nitrite Levels in Chicken Sausage Brands

The mean nitrite levels in the chicken sausage brands ranged from of 24.7± 1.4 in top ten chicken sausage to 335.67 ±16.54 in imperial chicken brand (Figure 4.3). Analysis of variance (ANOVA) at 95% confidence level revealed that nitrite concentrations among the various chicken sausage brands differed significantly ($P<0.05$). However, when multiple comparism was conducted in means using the Tukey's HSD multiple comparism test, it revealed that there were no statistically significant differences among the following brands; top ten, malicat, jean caby and French polony. There were however different from Sadia, imperial, frankfuter, minibites and careplan brands. Among the brands French polony, topten jean caby malicat and master chicken recorded nitrite levels below the set standard (See appendix 3; Table 3.3).



Brands of chicken sausage

Figure 4.4: Mean nitrite levels of chicken sausage brands

4.3.1.4 Nitrite Levels in Pork Sausage Brands

The mean nitrite levels in the pork sausage brands ranged from a minimum of 60.82± 11.1 in pork sausage to a maximum of 242.1±2.7 in smokie pork (Figure 4.5). Analysis of variance (ANOVA) at 95% confidence level revealed that nitrite concentrations among the brands of pork sausage differed significantly (P<0.05). When the Least Significant Difference (LSD) was used to separate the means, it showed that there were differences among all the brands of pork products (Appendix 3; Table 3.5).

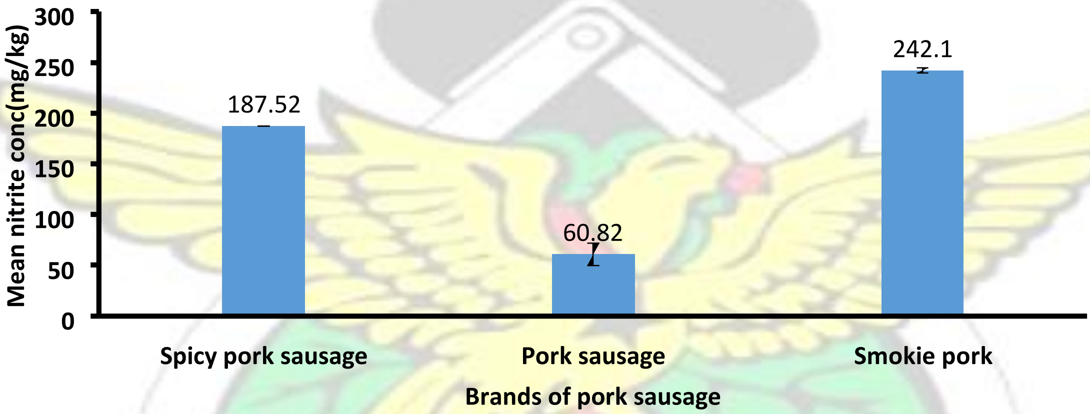


Figure 4.6: Mean nitrite levels of pork sausage brands

4.3.1.5 Nitrite Levels in Corned Beef Brands

The mean nitrite levels in the corned beef brands ranged from 15.4± 0.74 in lele brands to 203.1 ±0.00 in Exeter corned beef brand (Figure 4.4). Analysis of variance (ANOVA) at 95% confidence level showed that nitrite concentrations among the various brands of corned beef differed significantly (P<0.05). However, when multiple comparism was conducted to compare the means using the Tukey's HSD multiple comparism, it showed that there was no statistically significant differences between obaapa and Exeter corned beef. There were also no differences in nitrite concentrations among the following corned beef brands; Esther, Heinz, bull brand, zwan, African queen, grace and geisha. Exerter and obaapa brand showed nitrite above the set standard and the rest were below (Appendix 3; Table 3.4).

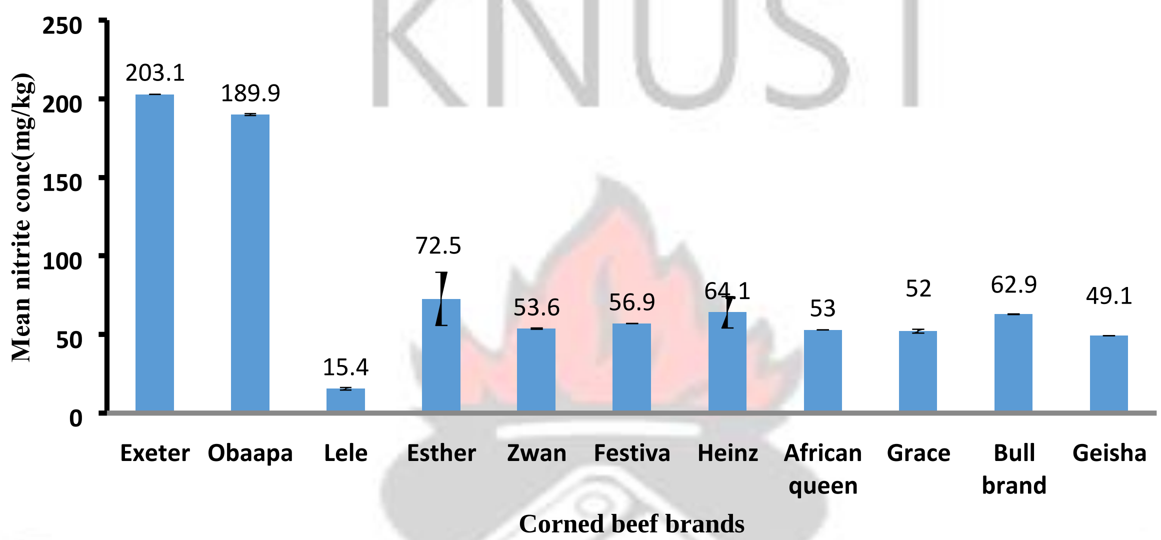


Figure 4.5: Mean nitrite levels of corned beef brands

4.3.2 Daily Nitrite Intake (Ingestion Rate per Day)

The mean daily nitrite intake among the study population was 115.15 mg/kg/day. That is, it can be said that, at a significant level of 5%, the population consume an average of 115.15 mg/kg bw/day of nitrite per body mass per day. Appendix 6 gives the minimum ingestion rate per day (5th percentile) as 0 mg/kg bw/day and the maximum ingestion rate (95th percentile) as 340 mg//kg/day at most times. The median ingestion rate (50th percentile) was 82.00 mg/kg bw/day whilst the mode was 0.00 mg/kg bw/day. The median ingestion rate of 82.00 mg/kg bw/day indicates that, the nitrite intake among 50% of the population is 82.00 mg/kg bw/day. The dietary intake of nitrite recorded in this study is far higher than that estimated by JECFA of FAO/WHO (0.0- 0.07mg /kg bw/day equivalent to 4.2 mg/ day for an individual weighing

60 kg.) for the general population (Mensinga *et al.*, 2003). Although other studies in other countries have recorded higher nitrite exposure rates which revealed an average nitrite uptake through diet in students as 1.7 mg/kg BW, as investigated by Wawrzyniak *et al*,(2009) but the dietary intake (115.15mg/kg bodyweight/day) in this study still remains higher. For example, in the Denmark a study in children indicated nitrite uptake of 0.014 mg/kg B.W due to meat products consumption (Leth *et al*., 2006). Larsson *et al*, reported that the nitrite uptake from the diet in Swedish children between 11 and 12-year-old was 0.007 mg/kg B.W. Also another study conducted by Reinik *et al*., (2005) at Stony in 346 children through the entire Estonian meat products revealed nitrite intake of 0.83mg/day. In their study, the average nitrite intake (0.83 mg/kg/day) was far higher than the average nitrite intake from EU foods (0.06 mg/kg/day) which even called for urgent action to minimize the level of nitrite in foods when it was reported by determining Nitrites, nitrates and *N*-nitrosoamines in Estonian cured meat products: Intake by Estonian children and adolescents. Comparative to this current studies still reveal higher nitrite intake among study population and immediate measures must be put in place. The high nitrite intake recorded in this study may be attributed to the maintenance time. In addition, the percentage of meat in the processed meat, processing, and poor storage conditions may be the other factors for the higher level of nitrite intake.

4.3.3 Mass of Processed Meat

Appendix 6 explains that, at a 90 % confidence interval, the mass of meat consumed range between 15 g and 125 g. The mean mass (g) of meat consumed per day is 58.80g. That is on the average, a person consumes 58.80 g of meat per day. The average consumer consumes approximately 58.80 g of meat per day, whiles at most times, the minimum mass (5th percentile) of meat consumed is 15 g and the maximum mass (95th percentile) of a meat for heavy consumers is 125 g. The median and modal of the meat recorded were 52 g and 38 g respectively.

4.3.4 Body Weight of the Study Population

The minimum body weight of the population was approximately 10 kg whilst the maximum body weight was approximately 135 kg (Appendix 6.). The most likely weight among the participants was 48 kg, mean body weight was 53.28kg and median weight was 52kg. A body weight of 30 kg was recorded for the participant with the least body weight (5th percentile) while the highest body weight (95th percentile) among the populace was 82 kg.

4.3.5 Number of Times of Consumption of Meat per Day

From Appendix 6, at a 90 % confidence interval, it can be said that, the number of processed meat consumed per day by the study population range between 0.00 and 10.00. The mean number of times of consumption meat per day is 1.9602 which is approximately, 2. That is, we are 90 % confident that at most times, a person averagely, consumed 2 portions of 58.8 g sized meat per day

4.3.6 Chronic Daily Nitrite Intake (CDI)

The mean CDI of nitrite per person in the study population is 5.05mg/kg/day, median CDI is 2.09 mg/kg/day (Appendix 6). That is, within the population the mean CDI of nitrite is 5.05mg/kg/day at most times. However, the CDI for the 95th percentile consumer is 19.85mg/kg/day and the 5th percentile consumer is 0.00mg/kg/day. It can further be explained that, the concentration of nitrite that a person ingest per day in order to stand a probable risk of adverse health effect within 1 year is 5.05mg/kg/day for the average consumer, and 19.85mg/kg/day for the 95th percentile consumer or the heaviest consumers and those who do not consume much are fee of adverse effect.

4.3.7. Dietary Nitrite Risk

Since cancer assessment is not assessed under the EPA IRIS program the emphasis was on their non-carcinogenic effect and the risk (hazard quotient) evaluated in the study resulted in a mean value of 15. 65 which means the hazard quotient (HQ) > 1 and therefore a potential risk of adverse ill effect on the population (Figure 4.6). At 90 % confidence interval, the probability of a person to the risk of adverse health effect due to the eating of processed meat products is between 0 and 54.40. That is, the less consumers of these products do not stand the risk of adverse effect in a year whiles about 55 out of 100 people who ingest the products stands a maximum risk of adverse health effect in year.

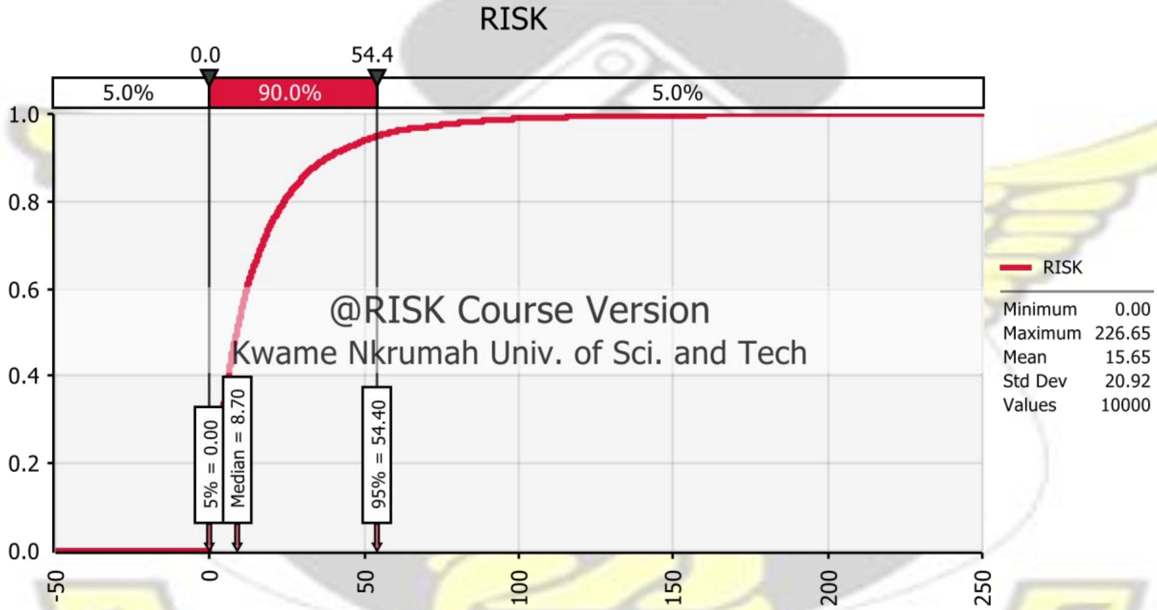


Figure 4.7: Daily consumption of processed meat and its nitrite risk after Monte Carlo simulation over 10,000 iterations

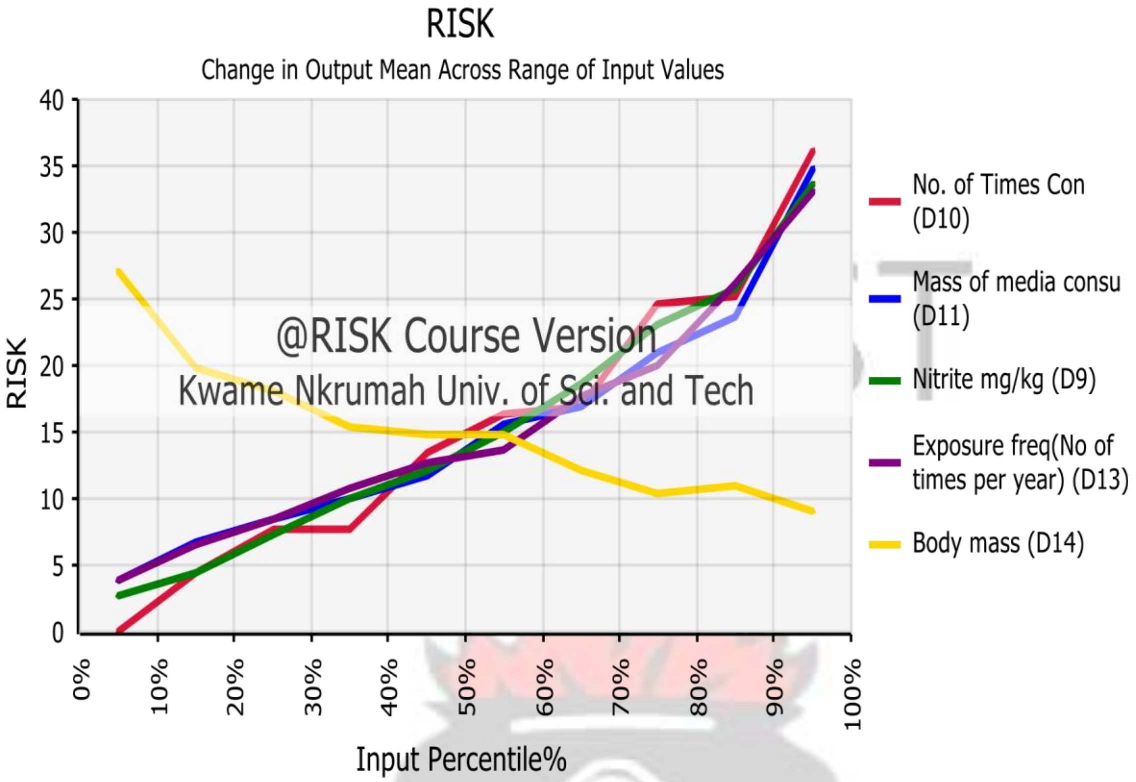


Figure 4.8: Changes in population risk to adverse health effect across range of input percentiles; exposure to nitrite, body weight, number of consumption of processed meat per day and mass of meat consumed per day.

4.3.8. Correlation between Risk and Input Parameters

Correlation between nitrite and risk: Figure 4.7 gives a summary of the correlation between nitrite content and its risk. It can be observed that before the 90th percentile, the risk is increases as the nitrite level increase even before the 90th percentile. There is a great increase in risk just before and after the 90th percentile. From this result, it can be said that, as the nitrite content in meat increases, a consumers risk to adverse health effect also increases. That is the higher consumer will tend to consume more nitrite and have a higher possible risk to ill effects associated with nitrite consumption such methemoglobinemia, mainly in children, and nitrite intoxication which causes vasodilatation, smooth muscle relaxation, face redness, gastrointestinal discomfort, headache, cyanosis and vomiting.

Correlation between number of meat consumed per day and risk: From Figure 4.7, there is gradual increase in risk for consumers of meat before the 90th percentile. The risk tends to increase steadily right before the 90th percentile as the number of meat consumed by a person increases the risk also increases. That is an increase in risk of persons who consume large number of processed meat per day than people who consume fewer amounts per day as well as those who consume in moderation. This confirms the saying that ―every substance is poisonous but its effect can be minimized if taken in small quantities.

Correlation between Exposure frequency and risk: As the number of time with respect the quantity of processed meat consumed by the population increase within a period the risk of adverse health effects also increases

Correlation between body weight and risk: As the body weight of the study population increases, risk decreases (Figure 4.7). This means that, people with larger body weight who consume processed meat from the study area have a lesser risk of getting adverse health effect than those with small body weight.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Nitrites and nitrates are two important additives in meat industry because of their beneficial effect on the quality and microbiological safety in meat products. On the other hand, the major concern of nitrates/nitrites in meat products is related to the potential of nitrites to form carcinogenic N-nitroso compounds. The findings revealed that commonly consumed processed meat products among Ghanaians in the Ayawaso West Sub Metro contained higher amount of residual nitrite (139.85 mg/kg) than permitted by WHO/EU standard of 125 mg/kg of nitrite. This high level of nitrite indicates that processed meat products contribute to the nitrite exposure of people. In addition, the estimated mean total dietary intake of nitrite in the population, from the consumption of corned beef, sausage and bacon based on the food frequency questionnaire was higher than other studies. The estimated risk value shows that consumers of sausages, corned beef and bacon are at a greater disadvantage to range of health risks. Thus, the probability of 50% of the study population who consume sausages, corned beef and bacon obtained from the Ayawaso West Sub Metro in the Accra metropolis to range of health risk within a year is about 9 out of every 100 people.

5.2 Recommendations

The study conducted chose respondents of all categories of age, region and social class and therefore recommend that further evaluation must focused on more vulnerable groups such as children and pregnant women with possibly high consumption of certain products like sausages.

It is also recommended that further work be done in the same study area which will focus on the levels of nitrosamine to evaluate the carcinogenicity of processed meat product sold on the

Ghanaian markets. On the basis of the current results, efforts should be pursued by the general population to reduce exposure to nitrite by both changing food habits and lowering contents of cured meat product in their diets. The regulatory bodies like the Food and Drug Authority must educate the public on the amount of nitrite that is permissible as an additive and also monitor the use of nitrite in local production as well as imported ones. The FDA must as well educate producers to change production technologies, decrease levels of ingoing nitrite and wider use of cure accelerators such sodium or potassium ascorbate and erythobate. Finally, the risk results of this study calls for the concern of government and public health officials to put in measures on how to reduce the risk of consumers of processed meat products to adverse health effect as well as certain human cancers.

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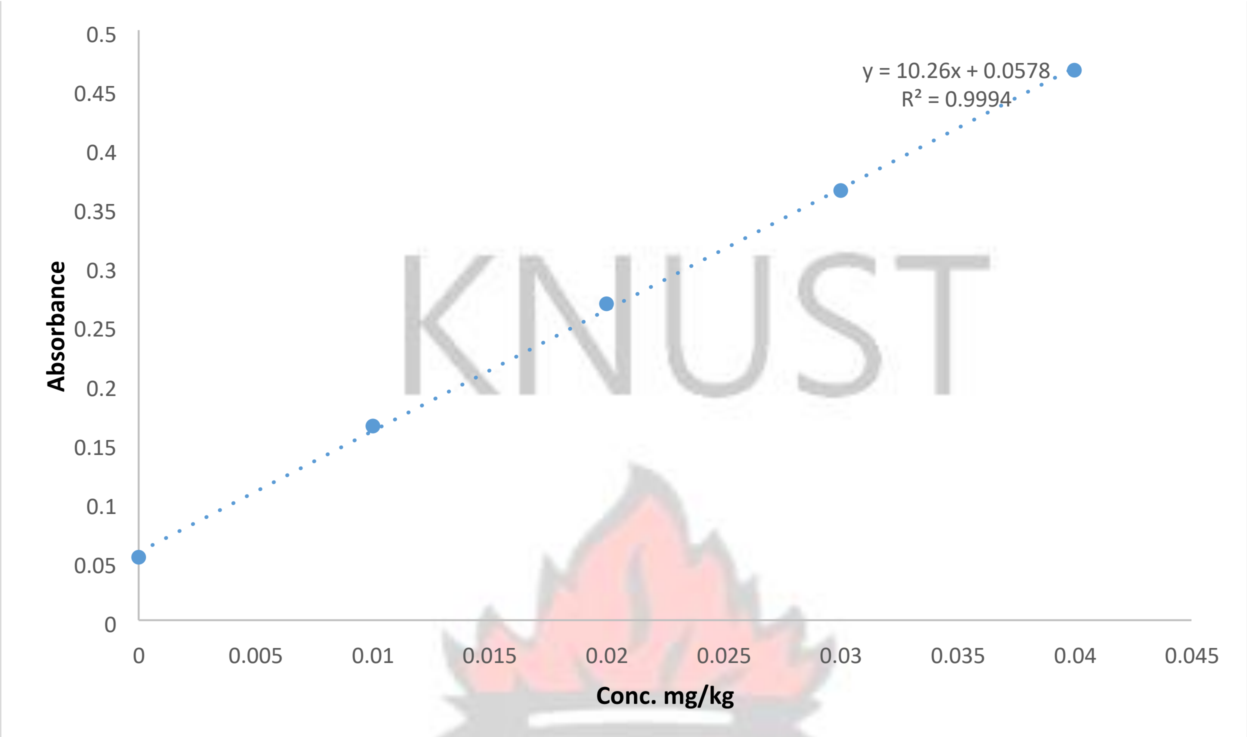
APPENDIX

APPENDIX 1

NITRITE STANDARD CURVE FOR DETERMINING THE CONCENTRATION OF NITRTE CONCENTRATION IN SAMPLED PROCESSED MEAT PRODUCTS



APPENDIX 2

CONCENTRATION OF NITRITE IN SAMPLED PROCESSED MEAT PRODUCTS

Table 1: The Results of nitrite concentration (mg/kg) in the processed meat Samples

Sample Name	Nitrite Concentration mg/kg
Back Bacon	38.40156
Hind Bacon	46.19883
Streaky Bacon	42.30019
Bitso Bacon	39.37622
Collar Bacon	49.12281
Spicy Pork Sausage	187.5244
Porky Sausage	60.81871
Smokie Pork	242.1053

Beef Kheba	259.6491
Dannka	147.5634
Emborg	242.1053
Beef Smokie	343.4698
First Sausage	86.15984
Tropican Beef Sausage	127.0955
Meaty Beef	77.76803
Nempa Sausage	253.8012
Adom Sausage	100.7797
Bino Sausage	186.5497
Beef Sausage	236.2573
Beef Sausage	187.5244

Beef Sausage	181.6764
Beef Sausage	259.6491
Beef Sausage	147.5634
Beef Sausage	242.1053
Chicken Sausage	186.5497
Sadia	236.2573
Careplan Sausage	141.7154
Minibites	181.6764
French Polony	30.60429
Imperal	335.6725
Topten Sausage	24.75634

Jean Caby	45.22417
Malicat	29.62963
Master Chicken	188.499
Chicken Sausage	245.0292
Frankfurter	137.3687
Chicken Sausage	55.94542
Chicken Sausage	6.288499
Chicken Sausage	86.15984
Exerter	203.1189
Obaapa	189.9095
Lele	15.45995
Esther	82.26121
Zwan	53.52688
Festiva	56.92008
Heinz	69.14561
African Queen	53.02144
Grace	52.04678
Bull Brand	62.98463
Geisha	49.12281

Mean 139.85

Median 124.74

Std Dev. 87.96

APPENDIX 3

COMPARING NITRITE LEVELS IN VARIOUS BRANDS OF PROCESSED MEAT

Table 3.1: BACON SOURCE

DESCRIPTIVE STATISTICS

Nitrite concentrations

Bacon sources	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
back bacon	2	38.4016	6.89188	4.87329	-23.5195	100.3226	33.53	43.27
streaky bacon	2	46.1988	1.37838	.97466	33.8146	58.5830	45.22	47.17
Ent.bacon	2	42.3002	1.37838	.97466	29.9160	54.6844	41.33	43.27
bitso bacon	2	39.3762	2.75675	1.94932	14.6078	64.1446	37.43	41.33
collar bacon	2	49.1228	2.75675	1.94932	24.3544	73.8912	47.17	51.07

Total	10	43.0799	5.07072	1.60350	39.4525	46.7073	33.53	51.07

ANOVA

Nitrite

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	164.913	4	41.228	3.100	.043
Within Groups	66.497	5	13.299		
Total	231.410	9			

Table 3.2: BEEF SAUSAGE

DESCRIPTIVE STATISTICS

Nitrite

Beef sausage	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
beef khebab	2	259.6491	2.75675	1.94932	234.8807	284.4176	257.70	261.60
Dannka	2	147.5634	6.89188	4.87329	85.6423	209.4844	142.69	152.44
Emborg	2	242.1053	2.75675	1.94932	217.3368	266.8737	240.16	244.05
beef smokie	2	343.4698	11.02701	7.79727	244.3961	442.5435	335.67	351.27
First sausage	2	86.1598	.00000	.00000	86.1598	86.1598	86.16	86.16

Tropican beef sausage	2	127.0955	.00000	.00000	127.0955	127.0955	127.10	127.10
Meaty beef	2	62.7680	19.29726	13.64522	-110.6110	236.1470	49.12	76.41
Nempa sausage	2	253.8012	5.51350	3.89864	204.2643	303.3380	249.90	257.70
Adom sausage	2	100.7797	9.64863	6.82261	14.0902	187.4692	93.96	107.60
Bino sausage	2	186.5497	1.37838	.97466	174.1655	198.9339	185.58	187.52
Beef sausage Total	12	209.1293	41.38850	11.94783	182.8323	235.4263	142.69	261.60
	32	191.5448	75.26738	13.30552	164.4081	218.6816	49.12	351.27

ANOVA

Nitrite

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	156095.354	10	15609.535	16.789	.000
Within Groups	19525.159	21	929.769		
Total	175620.514	31			

Table 3.3: CHICKEN SAUSAGE

DESCRIPTIVE STATISTICS

Nitrite

Chicken sausage	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Frankfurter	15	137.3687	118.37986	30.56555	71.8121	202.9253	6.24	364.91
Sadia careplan sausage	2	236.2573	.00000	.00000	236.2573	236.2573	236.26	236.26
	2	141.7154	34.45939	24.36647	-167.8900	451.3208	117.35	166.08
Minibites	2	181.6764	2.75675	1.94932	156.9080	206.4448	179.73	183.63
french polony	2	30.6043	1.37838	.97466	18.2201	42.9885	29.63	31.58
	2	335.6725	16.54051	11.69591	187.0619	484.2831	323.98	347.37
Imperal topten sausage jean caby	2	24.7563	1.37838	.97466	12.3721	37.1406	23.78	25.73
	2	45.2242	5.51350	3.89864	-4.3127	94.7610	41.33	49.12
Malicat	2	29.6296	.00000	.00000	29.6296	29.6296	29.63	29.63
master chicken Total	2	121.2476	.00000	.00000	121.2476	121.2476	121.25	121.25
	33	131.9423	110.08120	19.16268	92.9092	170.9754	6.24	364.91

ANOVA

Nitrite

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	190075.953	9	21119.550	2.457	.040
Within Groups	197695.930	23	8595.475		
Total	387771.883	32			

Table 3.4: CORNED BEEF

DESCRIPTIVE STATISTICS

Nitrite

Corned beef	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Exerter	2	203.1189	.00000	.00000	203.1189	203.1189	203.12	203.12
Obaapa	2	189.9095	.61633	.43581	184.3720	195.4470	189.47	190.35

Lele	2	15.4599	.74170	.52446	8.7961	22.1238	14.94	15.98
Esther	3	72.5146	16.88159	9.74659	30.5784	114.4508	53.02	82.26
Zwan	2	53.6362	.56025	.39616	48.6025	58.6698	53.24	54.03
Festiva	2	56.9201	.00000	.00000	56.9201	56.9201	56.92	56.92
Heinz	2	64.1456	10.02522	7.08890	-25.9274	154.2186	57.06	71.23
African Queen	2	53.0214	.00000	.00000	53.0214	53.0214	53.02	53.02
Grace	2	52.0468	1.37838	.97466	39.6626	64.4310	51.07	53.02
Bull brand	2	62.9846	.30632	.21660	60.2325	65.7368	62.77	63.20
Geisha	2	49.1228	.00000	.00000	49.1228	49.1228	49.12	49.12
Total	23	79.0555	57.24403	11.93621	54.3013	103.8096	14.94	203.12

ANOVA

Nitrite

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	71417.625	10	7141.762	127.206	.000
Within Groups	673.719	12	56.143		

Total	72091.344	22				

Table 3.5: PORK SAUSAGE

DESCRIPTIVE STATISTICS

Nitrite

Pork sausage	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Spicy pork sausage	2	187.5244	.00000	.00000	187.5244	187.5244	187.52	187.52
Pork sausage	2	60.8187	11.02701	7.79727	-38.2550	159.8924	53.02	68.62
Smokie pork	2	242.1053	2.75675	1.94932	217.3368	266.8737	240.16	244.05
Total	6	163.4828	83.34027	34.02352	76.0225	250.9430	53.02	244.05

ANOVA

Nitrite

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	34598.807	2	17299.403	401.706	.000

Within Groups	129.195	3	43.065		
Total	34728.001	5			

Table 3.6: CONSUMPTION RATE OF PROCESSED MEAT PER DAY

	Percentage frequency				
Sample ID	**Once**	**Twice**	**Thrice**	**More Than Thrice**	**Never**
Corned Beef	15.4	23.40	50.70	9.50	1.0
Sausages	25.0	40.2	24.5	8.70	1.6
Bacon	20.0	32.5	22.8	24.0	0.7
Salami	15.8	7.40	1.30	0.50	75.0
Hot Dogs	15.60	5.40	4.80	0.50	70.9
Ham	22.80	10.6	1.6	0.50	64.50

APPENDIX 4

Table 4.1: Socio-Demographic Characteristic of Respondents

Demographic characteristics	**Female n (%)**	**Male n (%)**	**Total n (%)**
Sex	150 (57.7)	110(42.3)	260 (100.0)
Age (years)			
Mean age ± Standard Deviation	25±2.3	22.8±2.4	20.39 ± 1.6

Education level			
No formal education	6(2.3)	8(3.1)	14 (5.4)
Primary/JSS	30(11.5)	10(3.8)	40(15.4)
SHS	85(32.7)	35(13.5)	120(46.2)
Tertiary	50(19.2)	30(11.5)	80(30.8)
Religion			
Islamic	4 (1.5)	6(2.3)	10 (3.8)
Christianity	138 (53.1)	110 (42.3)	248 (95.4)
None	1 (0.38)	1(0.38)	2 (0.77)

APPENDIX 5

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY – COLLEGE OF SCIENCES KNUST

QUESTIONNAIRE ON CONSUMPTION RATE/ PATTERN AND CONSUMER PERCEPTION OF PROCESSED MEAT PRODUCTS

1. Age : Sex: female ( ) Male ( )

2. Weight(kg)______________ Height (m)______________________

3. Level of education:

Primary	secondary	tertiary	Others (specify)

4. Religion:

None	Traditional	Muslim	Christian	Other (specify)

/) **appropriately as many**

5. How often do you eat the following processed meat (**as**

you eat

	Once	Twice	thrice	More than thrice	Never	Quantity(g)
Sausage						
Bacon						
Corned beef						
Hot dogs						
Ham						
Salami						

6. From scale of 1 to 6, one (1) being the most preferred and six (6) the least preferred please rank the most preferred among the list.

Sausage ______ bacon _________ corned Beef _________ Salami _________

Hot dogs _______ Ham ___________

7. State where you normally buy your processed meat from?

____

8. What brand of the processed meat do you prefer?

Locally produce	Imported

9. Please indicate how you agree with the following statements;
(1) Strongly Disagree (2) Disagree (3) Neutral (4) Agree (5) Strongly agree

Expectations Of Good Processed Meat	**1**	**2**	**3**	**4**	**5**
The colour of a good processed meat should be brightly brick red					
Good processed meat should produce meaty-like flavour					
All spices must be tasted in the meat					
The meat must not taste rancid					

10. (a.) Do you at times observe any changes after purchasing/consuming a processed meat?

☐ Yes

☐ No

(b). If yes what was/ were the issues

________Change in colour

________Change in taste

________Change in texture

________Health implications

APPENDIX 6

RESULT OF GRAPHS AFTER MONTE CARLO SIMULATION OVER 10,000 ITERATION

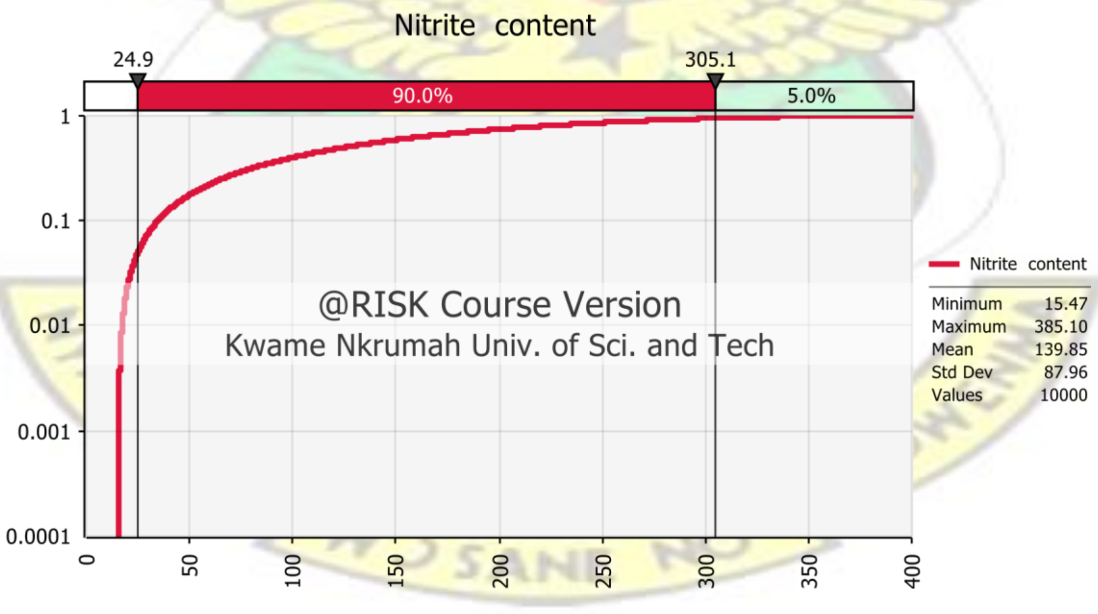


Figure 6.1: log logistic distribution of nitrite content in processed meat samples obtained from the Ayawaso West Sub Metro based on a first order Monte Carlo simulation.

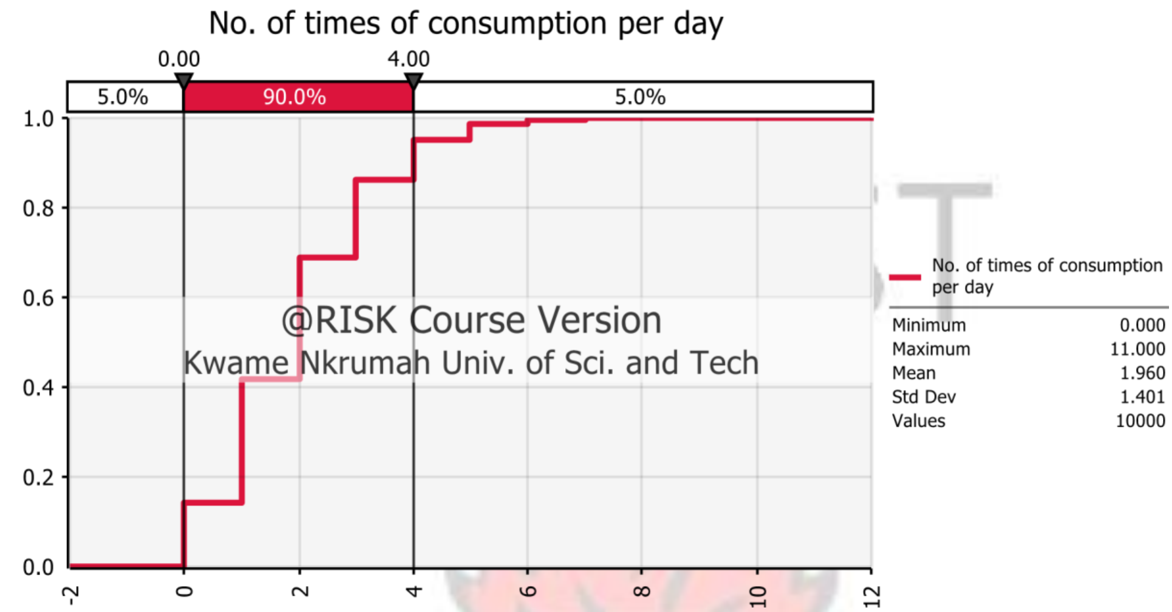


Figure 6.2: Histogram distribution for the number of meat consumed per day by the study population after Monte Carlo simulation over 10,000 iterations

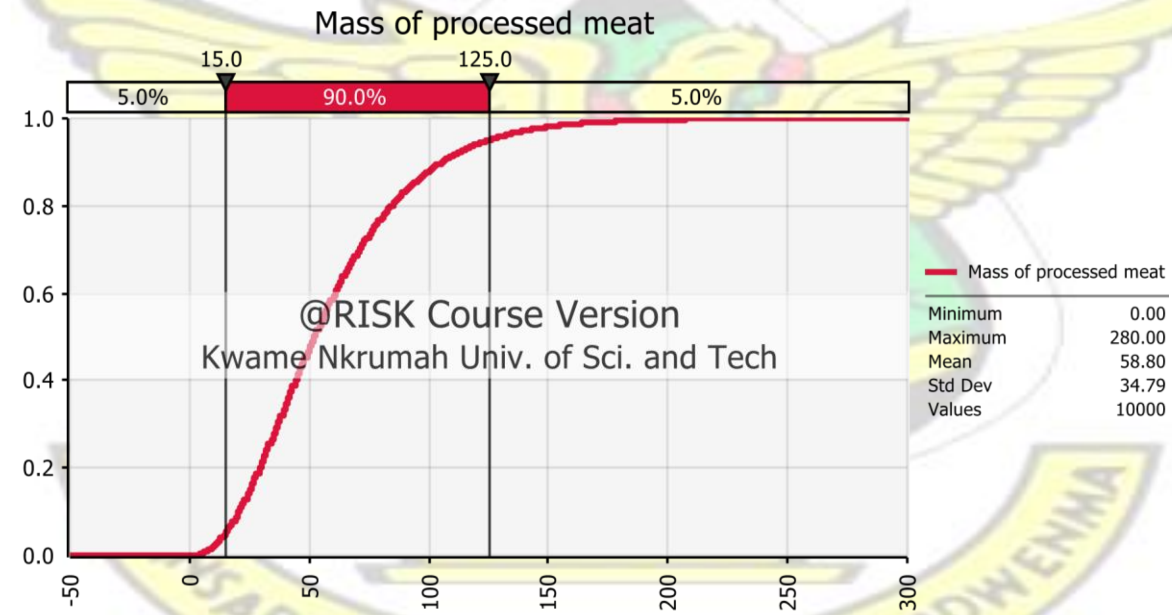


Figure 6.3: Uniform distribution representing the first order Monte Carlo simulation of the mass of meat eaten among the study population

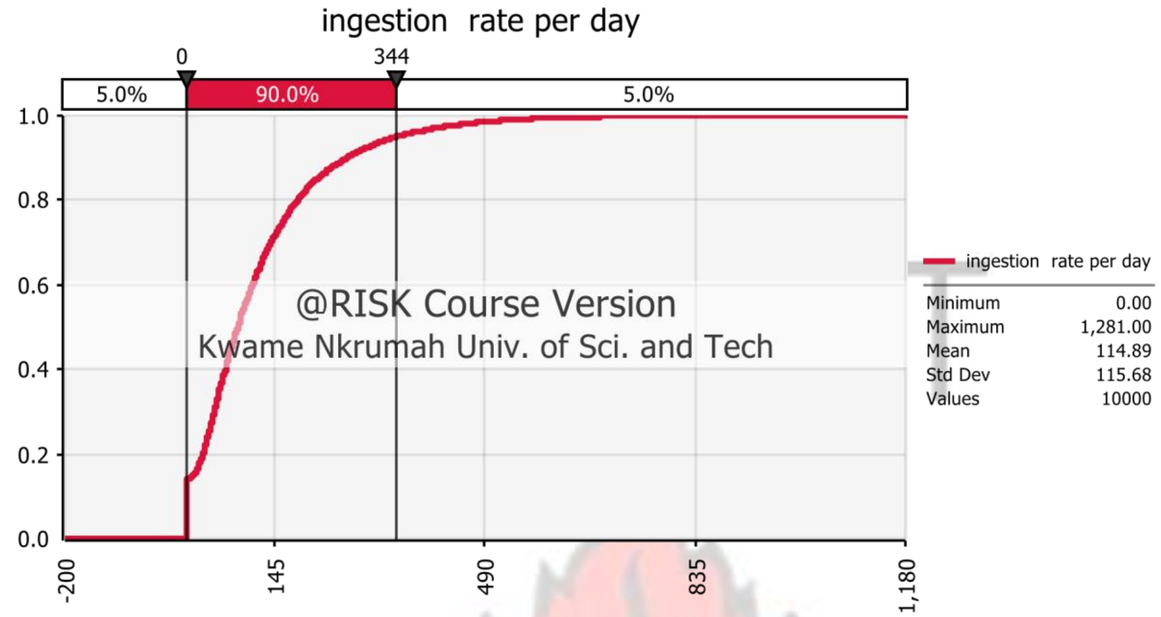


Figure 6.4: Distribution of the daily dietary nitrite intake of meat among the populace

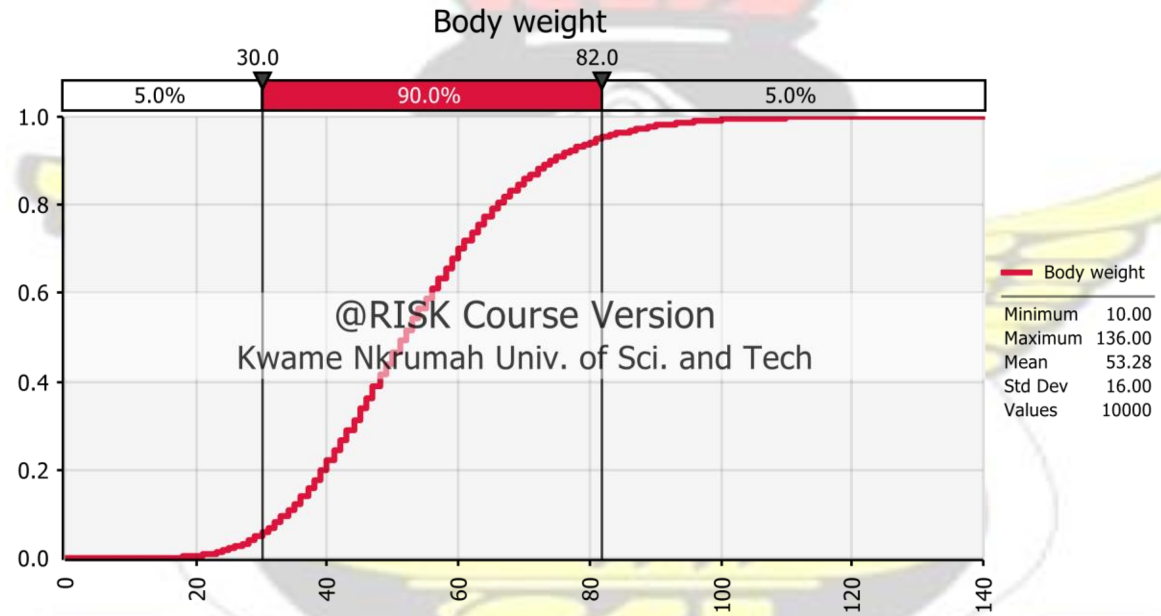


Figure 6.5: Histogram distribution representing the Monte Carlo simulation of the body weight of the study population

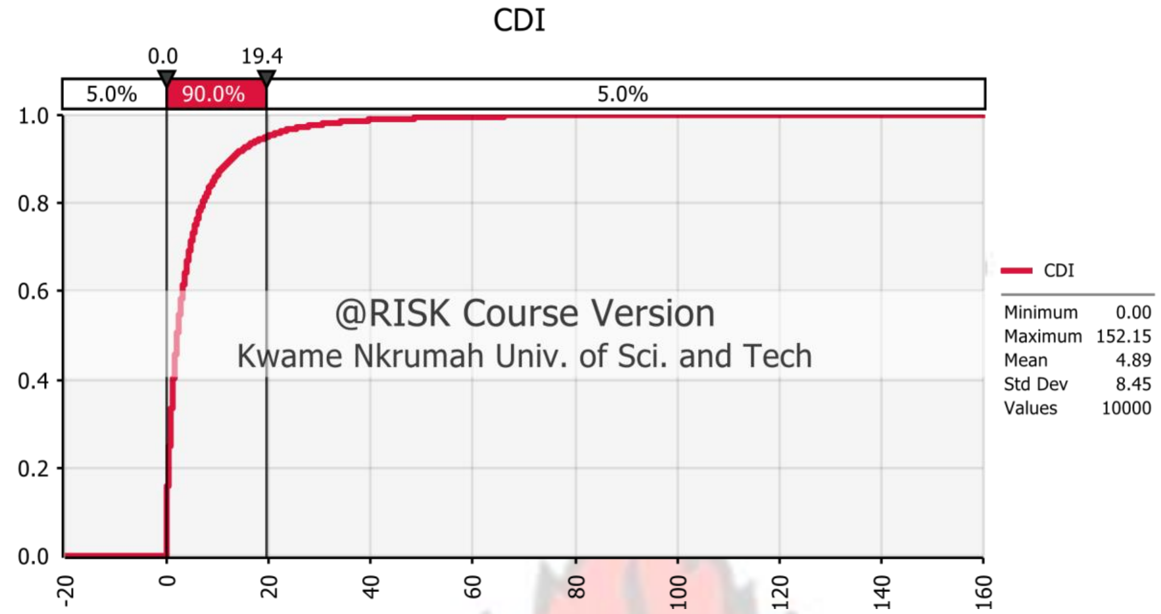


Figure 6.6: Chronic daily intake of nitrite after Monte Carlo simulation over 10,000 iterations