KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

EFFECT OF COOKING METHODS ON RESIDUAL NITRITE LEVELS IN COMMONLY CONSUMED SAUSAGE 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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DECLARATION

I hereby declare that this submission is the result of my own research work towards my MSc degree and that, to the best of my knowledge; it does not contain any previously published material except for some information which the source for each one has been duly acknowledged and stated clearly.

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DEDICATION

This work is sincerely dedicated to the Almighty God and my wonderful and supportive family, especially my cherished wife Mrs. Mercy Johnson Odei Antwi, my children Anna Appiah Odei Antwi and Emmanuella Odei Antwi.

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ABSTRACT

Sausages are a processed form of meat popular in Ghana, especially among children. Sausages contain nitrite which serves as a curing agent for most processed meat products and is used as

preservative, antioxidant and a colour fixative. It is a precursor of carcinogenic N-nitrosamines during the processing of meat products or through human digestion activities. This study aimed to investigate the effect of different cooking methods on the residual nitrite concentration of different brands of commercially available sausage in the Ashaiman market in the Tema district of the Greater Accra Region of Ghana. A total of 18 samples of six (6) different brands were used in this study. The study employed three (3) common cooking methods used in cooking sausage in Ghana; boiling, frying and grilling. Raw (uncooked) sausage served as the control. Determination of the residual nitrite concentration of the different sausage brands cooked using different cooking methods was by a spectrophotometric method. The residual mean nitrite concentration (ppm) for the raw, boiled, fried and grilled sausage brands were as follows; for brand A; 164, 78, 81 and 80; for brand B; 248, 218, 269 and 387; for brand C; 108, 104, 217 and 198, for brand D; 187, 154, 225 and 150, for brand E; 188, 54, 45,and 47, and for the last brand F; 347, 190, 218, and 205, respectively. The results revealed that brands A and E had significantly reduced nitrite concentration for all three cooking methods. Also, among the six (6) brands of raw sausage investigated, the nitrite concentration level of brand C (108 ppm) was within the set acceptable limit (125 ppm) by the Ghana Standard Authority and WHO/EU recommended levels with the rest showing values above the limit in processed meats. The results suggest boiling as a safe cooking method for cured meat and sausages found on the Ghanaian market.

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LIST OF ABBREVIATIONS

ANOVA……..Analysis of Variance

Brand A……German Vienna

Brand B……Nempa

Brand C……Adom

Brand D……Bino

Brand E……Frankfurter

Brand F……Imperial

CCMP……..Cooked Cured Meat Pigment

EU……European Union

GFDA……..Ghana Food and Drugs Authority

GHS……Ghana Health Service

GNA……Ghana News Agency

GSA……...……...Ghana Standards Authority

WHO……World Health Organization

IARC…….……International Agency for Research on Cancer

CHAPTER ONE

INTRODUCTION

1.1 Background

Processed meat is any edible part of an animal that is edible, which has been processed by salting, curing, fermentation, smoking, or any other process with the aim of preserving and enhancing the flavour. Processed meat includes products such as sausage, bacon, ham, chicken nuggets and more (IARC, 2015). Consumption of meat in developing nations and to some extent developed nations is on the rise and the rising demand is as a result of the fast progression of urbanization and the desire among city settlers to spend more on food than the lower income earning rural population. The desire for meat consumption by humans is ancient and has a biological connection. In ancient times meat was clearly the preferred choice borne out of availability and delicacy (Heinz and Hautzinger, 2007).

Processed meat and poultry are extremely perishable making them vulnerable for the action of microorganisms such as bacteria, yeast and moulds. This vulnerability is due to the high level of moisture they contain, the favourable pH level for microbial activity and the richness in proteins, peptides and amino acids. The microorganisms not only cause a decline in the nutritional quality of the meat products but also the sensory quality, thereby causing deterioration and limiting the shelf life. Besides these implications, they are responsible for human illness. The use of salt for meat preservatio prevents growth of some types of bacteria responsible for meat spoilage. The antimicrobial effect of salt has been found to be due to the direct inhibitory effect or the drying effect it has on meat since most bacteria require a substantial amount of moisture to live and grow. As salt use spread, preference for certain salts which not only have antimicrobial property but also

produce pink colour and special flavour developed. Nitrate evolved as it was found to possess these properties. Further studies showed that nitrate did not have a direct effect on meat colour but it was nitrite, the end product when nitrate is acted upon by bacteria during processing and storage (Epley *et al*., 1992).

Processed meats are products that have been changed from their original fresh state. Some have added ingredients like spices. Some are cooked and some are cured. Some are ready-to-cook whereas others are ready-to eat. Processed meats are commonly made from beef, pork, chicken and turkey and each of these offer high quality protein, vitamins and minerals. (Zhao *et al.,* 2007).

Sodium nitrite is a compound that is used to "cure" meats. Cured meats have a characteristic colour, unique taste and a long shelf life. Centuries ago, nitrate was used in the form of saltpeter to cure meats before refrigeration was available. This was especially important in preventing the growth of the *bacterium Clostridium* botulinum, which causes botulism. (Bryan *et al.,* 2012). In the 20th century, meat processors shifted to sodium nitrite because it was more reliable in its effects. Since sodium nitrite has been used in commercially prepared meats, no cases of botulism have been linked to these products in the U.S. (Bryan *et al.,* 2012). Although naturally present in foods such as vegetables, most discussion on nitrate and nitrite focuses on cured meats because of the deliberate addition of nitrate and nitrite to meat as a preservative, thereby raising considerable concerns whether the levels of nitrate and nitrite in cured meat is within the acceptable levels set by the competent authorities such as the

European Union (EU) and Ghana Food and Drugs Authority (GFDA).
Nitrite/nitrate is an approved food additive in the EU (EU Regulation No. 1129/2011/EC) widely used in meat preservation in the meat industry. The amount of permitted

nitrite/nitrate for use as a food additive in cured meat currently stands at 150 mg kg-1 (Govari *et al*, 2015). Nitrite has been widely accepted and used as curing agent for meat products due to its important favourable properties such as colour enhancement and anti-microbial effects (Archer, 2002). However, in the 1970s, a debate broke up on the formation of carcinogenic nitrosamines in meat products due to high levels of nitrite present (Sannino and Bolzoni, 2013). This resulted in strong pressure from government agencies, food activist and consumers to decrease the use of nitrite for curing, in order to reduce the risk of nitrosamine formation and associated health risks (Merino *et al.,* 2016)

The use of nitrite in meat and especially in sausage has been clouded by suspicions that, nitrite could react with amines in the gastric acid in the body and form carcinogenic nitrosamines which have the potential of causing various cancers. (Archer, 2002). Today, nitrites (potassium nitrite, KNO_2 and sodium nitrite, $NaNO_2$) are well known as curing agents in producing cured meat. As additives, they have been found to contribute to cured meat flavour, colour and also inhibit the growth of microorganisms, particularly *Clostridium botulinum*, and effectively control rancidity by inhibiting oxidation (Pearson and Gillett, 1996). Nitrates and nitrites are not only used in curing but are also present in several food products as naturally occurring compounds and as such they form part of human diet.

Sausage consumption in Ghana, particularly among children, is very much prevalent. It falls under the processed cured meats category of food products and it is made with pork, chicken or beef. It is highly preferred due to its exceptional taste, appealing colour and cost, making it available in rural and urban areas. This is due to the increasing number of fast food establishments. In addition, most food vendors in schools serve sausage as a

source of protein. Moreover, working class mothers prefer using sausage for breakfast due to its minimal cooking time.

It is estimated that, about 34,000 deaths from cancer every year could be due to diets with high intake of processed meat (Citi 97.3 FM, 2015). It is estimated that, about 16,600 cases of cancer occur annually in Ghana with an occurrence rate of about 109.5 cases per 100,000 persons as reported in Ghana by the Cancer Control Division of Ghana Health Service (GHS) on February 4, 2011. The report further stated that; most of the cases seen in Ghana and some West African countries identified the disease with younger people, which is in the direct opposite of what has been reported in the developed world (GNA, 2011).

In Another statement, the chief executive officer (CEO) of the non-governmental organization Breast Care International on March 22, 2011, stated 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 a contributing factor to the rising number of the reported cancer cases, especially breast cancer among Ghanaian women (GNA, 2011).

As part of cured meats products, sausage is also treated with nitrites, which gives it an improved colour, unique taste and a long shelf life. In addition, the sodium nitrite preserves it from microbial activities. However, studies show that nitrites use in processed meat as preservatives result in the formation of nitrosamines. Alahakoon, et al. (2015) stated that, due to the health problem posed by nitrites, and the rise in consumer demand for natural meat products, meat industries are focusing on the development of

nitrite alternatives. The study by Eskandari et al. (2013) focuses on alternate ways to reduce nitrite use, such as nitrite free and low nitrite meat curing systems using natural colourants. Their findings revealed that meat treatment with 0.015% cochineal, most closely resembled 120ppm $NaNO_2$ in producing cured meat colour. Also, N-nitroso compounds formed from the reactions of nitrite with amines in meat are considered mutagenic and teratogenic (Essumang *et al.,* 2012). The risk of colorectal and stomach cancer increases with intake of red meat and processed meat (nitrite-preserved) according to epidemiologic studies, with processed meat showing higher risk estimates per gram of intake than red meat (Essumang *et al.,* 2012).

Even though, butylated hydroxylanisole (BHA) was found to be a strong antioxidant at 30ppm level in cooked sausages during storage, no single compound has yet been found to perform the nitrite function in meat including improved colour, taste and antimicrobial property. Due to this, nitrate and nitrite use as additives in the meat industry is still ongoing but with controlled levels of application (Hospital *et al.,* 2015).

Nitrosamine formation is linked to nitrate and nitrite use especially in cured meat products. It is a powerful and potent carcinogen with possible mutagenic and teratogenic properties (Eichhoizer and Gutzwiller, 1998). Studies show its presence in cigarette smoke. Due to sausage consumption by children, nitrosamine has been associated with two leading paediatric cancers; brain tumors and childhood leukemia.

Nitrosamine formation occurs when amines that occur naturally in food react with sodium nitrite found in cured meat products.

R_2NH (amines) + $NaNO_2$ (sodium nitrite) → $R_2N–N=O$ (nitrosamine)

N-nitroso compounds (NOC) are potent carcinogens and the epidemiological study by Eichhoizer and Gutzwiller (1998) showed inconclusiveness in the carcinogenic potential of dietary NOC and precursor nitrates and nitrites in human remains with regard to the risk of stomach, brain, esophageal, and nasopharyngeal cancers. Further epidemiological studies showed a positive relationship between cancer incidence and high intake of processed meat since N-nitrosamines (NAs) are a potential causative factor (Herrmann *et al*, 2015). Nitrates used in cured meat products are converted to nitrites in the gut and saliva by bacterial nitrate reductase increasing the risks of cancer and the more consumed processed meat, the greater the health risk, such as cancer
(Herrmann *et al*, 2015).

Even though nitrite may be harmful, studies prove an economic importance in adult gastrointestinal and cardiovascular physiology. Kanady *et al.* (2012) stated that swallowed nitrite is converted to nitric oxide (NO) in the stomach, which exerts protective effects in the gastrointestinal tract and throughout the body.
Genotoxic compound formation has been associated with consuming overcooked processed meat (Van Hecke *et al*., 2015). Further study by Li *et al.* (2012) showed significant increase in the concentrations of volatile N-nitrosamines when processed meat was deep-fried or pan-fried compared to when it was boiled or microwaved. Li *et al.* (2012) added that there was no significant difference after the boiling and microwave treatments compared to the raw due to the significant decrease in the total biogenic amines. The results suggested that boiling and microwave treatments were suitable methods for processing meat products. This was because residual nitrite was significantly reduced. Herrmann *et al.* (2015) added that levels of some volatile Nnitrosamines

increase after frying and baking of processed meat whiles the levels of non-volatile nitrosamines decreased.

In view of the known health risk posed by the use of nitrate and nitrite in meat products, industries should focus on minimizing its use in preserved foods to the minimum required to ensure microbiological safety. In addition, focus should be on the use of inhibitors of nitrozation such as α-tocopherol or ascorbic acid as proposed by
Walker (2009).

1.2 Problem Statement and justification

Sausage as a meat product is a major source of protein and other essential nutrients which are consumed by people of Ghana and worldwide. The processing of sausage involves the use of nitrite and nitrate as a preservative for curing purposes to inhibit the growth and production of *Clostridium botulinum*, a toxin-forming bacterium and a harmful pathogen found in stored meat products (Joannou *et al*., 1999). It is estimated that 16,000 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. Sausage consumption is more prevalent in children than in adults and this could be alarming due to the formation of N-nitrosamine which is carcinogenic in sausages consumed by these children who have weak immune system is a cause for worry. Several studies have shown works that tends to reduce the nitrite content at the production level by the quantities of addition (Honikel, 2008). Also, the effect of cooking methods on the nitrite content in cured meat with vegetables, which indicated a reduction in nitrite content, has been reported (Ya *et al*., 2016).

However, few studies focused on the effect of cooking methods on the nitrite content in sausage. (Ya *et al*., 2016). Therefore, it is important to study and know the levels of nitrites in some commercially commonly sold sausage and how three (3) different cooking methods (boiling, frying and grilling) affect these nitrite levels of sausage commonly sold on the Ashaiman community of Tema district in Greater Accra, Ghana. Moreover, little is known about the levels of nitrites in cured meat products in Ghana.

The study will inform the Ghanaians and the regulatory bodies such as Ghana Food and Drugs Authority (GFDA) and Ghana Standards Authority (GSA) on the brands of sausages commercially sold in the market that could pose threats to consumers' health and the effect some cooking methods on them. It will also inform the Ministry of Health and the health regulatory bodies such as Ghana Health Service (GHS) on the possible causes of paediatric cancer since it is on the rise. Researchers will be informed to do more on finding alternatives to nitrates and nitrites in the cured meat processing due to the formation of carcinogenic nitrosamines.

1.3 Objective of the study

The main objective of this study was to investigate the effect of three (3) cooking methods on residual nitrite levels in some selected commercial sausage products commonly sold and consumed in Ghana.

The specific objectives were:

1. To determine the residual nitrite levels in fresh raw sausages sold on Ghanaian market and compare to the acceptable standard level.
2. To assesses the effect of cooking methods on the residual nitrite level.

This research therefore seeks to establish the fact that the residual nitrate/nitrite levels are within the acceptable limit before consumption and does not pose any health concerns for consumers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat

Animal flesh which serves as food is called meat. Meat has a high nutrient composition that promotes the wellbeing of mankind (Zhou *et al.,* 2010). Red meats are full of iron, an element which is needed by the body to build and maintain blood haemoglobin, 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).

2.2 Meat Preservation

The main source of animal protein for many people all over the world is meat. It was reported that about 3.6 billion kg of meat and poultry ends up in the waste bin along the line of the consumer, retailer and food service levels which has a great impact on the economy. Most of the cause of this lost is largely due to microbial activities (spoilage) (Dave and Ghaly, 2011). According to Cerveny *et al.* (2009) about 5% of the meat and poultry loss incurred could satisfy the daily protein needs of approximately 320,000 people. Production of meat involves a series of operational steps which at each stage needs to be hygienically acceptable to reduce the amount of loss from microbial activities. It is therefore necessary to develop and maintain optimum preservation technique to keep these products safe (Dave and Ghaly, 2011).

2.3 Meat Preservation methods

Meat, rich in protein makes it an ideal hub for microbial and pathogenic growth and thereby necessitates meat preservation. Meat preservation methods mainly inhibit microbial growth, however other methods reduce deteriorative changes such colour and oxidative changes. Factors influencing the shelf life and quality of meat product include holding temperature, atmospheric oxygen (O_2), endogenous enzymes, moisture (dehydration), light and, most importantly, micro-organisms (Zhou *et al*., 2010).

Meat preservation methods commonly used rely on moisture control, temperature control and microbial loads control in the process line. Processed meats are highly perishable, and one of the main agents of their spoilage are microorganisms (bacteria, yeasts, and

molds). Microorganisms cause nutritional and sensory deterioration of meat products, bringing loss of quality and limiting shelf life. Apart from the economic implications of meat spoilage, microorganisms are also linked to human illness (Jiménez-Colmenero and Solana, 2007; Zhou *et al*., 2010).

Several preservation methods have been used to limit the speed and extent of meat spoilage and their consequences. Physical, chemical, and microbial methods of preservation have been used with the former being used traditionally and locally. Preserving processed meats by chemical means is by the use of additives known as preservatives. (Jiménez-Colmenero and Solana, 2007). Preservatives are known chemical compounds that, when added to foods, inhibit, retard, or prevent the activity and growth of spoilage and pathogenic microorganisms. Their main objective is 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 efficient use of preservatives, a control and regulation mechanism is much needed because these additives can have serious adverse health effects when wrongly prescribed or used. For consumer safety and protection the use of preservatives is subjected to strict a legal regulation regime, to create 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. (Jiménez-Colmenero and Solana 2007).

2.3.1 Smoking

Smoking, among the preservative methods is one of the oldest for meat preservation. This is done traditionally and locally with all or part of the meat placed over a wood fire. In

regions 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. The substances deposited on the meat contribute to the flavour and appearance but with ordinary, light smoking this preservative effect is limited and the product has to be stored refrigerated. Intensive smoking does prolong shelf life 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 (Heinz and Hautzinger, 2007).

2.3.2 Refrigeration

Refrigeration is a preservation method of maintaining the temperature of a particular environment lower than the surrounding environment. Food products are stored for several days or weeks under refrigeration temperatures of between 4-6^{o}C. Meat product can be preserved under this method as well as any other perishable products for longitivity and safety from microbial activities. Prolonged storage can lead to colour, taste and nutritional content deterioration (Bender, 1992).

2.3.3 Canning

The best form of meat storage is to put them into cans. 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 diets during all seasons irrespective of their geographical location.

2.4 History of meat curing

The preservation of meat by curing has been practiced for a long time and salt usage has been carried out for centuries. In the past, the Greeks used salt to preserve fish whilst the Romans preserved fish and meat with pickles containing salt and other ingredients (Pegg and Shahidi, 2000). With the long use of salt as a preservative, there has been the preference to improve the quality of meat products in terms of its pink colour, taste and shelf life rather than the less attractive grey colour and the short shelf life which existed. Nitrate impurities in rock salts were found to improve the pink colour and also give a special flavour to the meat product since it results in nitrite formation by the reducing activity of the muscle post-mortem (Pegg and Shahidi,
2000).

After this development, curing evolved and the need to improve meat products for consumption was solved, and meat curing was understood to include the use of saltpetre. By the end of the nineteenth century, various methods of curing such as dry cures, wet cures and combinations of both had been developed. (Toldra, 2002).

With dry curing, the cure ingredients are rubbed over the surface of the meat and over time, the curing ingredient get solubilized in the natural moisture of the muscle tissue (Toldra, 2002). With this process of curing, penetration of the cure into the meat is slow and usually more than one application of cure salts is needed. Furthermore, the meat is hung up in a cool dry atmosphere and moisture is slowly lost from it between treatments. The product is further subjected to a sequence of restacking which helps complete the cure, after which excess cure is washed off. The dry meat is then refrigerated and later dried again to evenly distribute the cure and ripen the product. Parma ham processing

uses the dry curing technique and it is tightly controlled and regulated (Toldra, 2002). Furthermore, the study stated that there are strict control measures throughout the process to ensure product safety.

Wet curing, also known as immersion curing involves putting the whole cuts of meat in brine solutions containing curing agents, and some traditional products. Modernization has resorted to the use of a perforated needle to inject meat with brine. This has greatly reduced the length of time required to cure products and multi-needle injector machines are now commonly used to produce cured products. Some producers have resorted to the use of arterial pumping which involves the distribution of the cure through the arteries of the meat, and also stitch pumping where the meat muscle is injected with a single needle and the natural channels present in the tissue help distribute the cure.
(Toldra, 2002).

2.5 Processed meat products

The growing consumer interest in „ready- to- eat" meat products such as some brands of sausages has led to higher rate of importation of the meat products which saw Ghana importing 1,362 metric ton (MTs) of sausages in 1999 jumped to an increase of 159 percent over the imports in 1998, Ghana Investment Promotion Centre (GIPC) (2000). The trend shoots up from 3063 MTs in 2008 to 3285 MTs in 2009, African Development Bank (ADB) (2011).

Worldwide, the intake of meat products is high and still rising most especially in developing countries (Cawthorn *et al.,* 2013). Ghana is ranked the highest consumer of meat products among sister African countries, followed closely by South Africa

(Cawthorn *et al.,* 2013). In Ghana, the commonest meat products include: sausages, bacon, ham, corned beef, hot dogs, salami etc.

2.6 The role of nitrate and nitrite

Nitrate used for meat curing, whether sodium or potassium nitrate was found to be converted to nitrite by nitrate-reducing bacteria (Gray and Pearson, 1984). They further explained that it was nitrite that was responsible for the increased colour change and that just small amounts are needed. Aside the colour effect it gives, nitrite also confers anti-microbial properties and other curing effects on meat (Jones and Betts, 2009).

Residual nitrite concentration can result in the formation of N-nitrosamine. Due to this, nitrate is no longer used in most curing processes to control residual nitrite concentration since it is more difficult to control the amount of nitrite formed
(Sebranek and Bacus, 2007).

Aside residual nitrite resulting in the formation of N-nitrosamine which is carcinogenic, Gray and Pearson (1984) stated that nitrite has many important functions in meat curing including improving cured meat colours, flavors and also inhibiting microbial growth which in turns slows rancidity. Studies have shown that the chemistry of nitrite in cured meat is a complex issue since nitrite is a very reactive compound, reacting with a wide range of substrates (Sebranek and Bacus, 2007). Pegg and Shahidi (2000) give a more comprehensive review of the issue.

2.7 Cured meat colour

Myoglobin is the pigment responsible for meat colour and this is dependent on the amount of myoglobin present in the meat and the chemical status of the pigment. It can be found in three forms which is, purple-red colour in raw meat, bright red when oxymyoglobin is involved and brown when it is metmyoglobin. The form is dependent on whether the pigment has been oxidized or reduced (Pegg and Shahidi, 2000). They further explained that, oxymyoglobin formation is from the oxygenation of myoglobin whilst metmyoglobin is from oxidation of the myoglobin.

The red colour of raw cured products is due to the presence of nitrosylmyoglobin which is formed from the reaction of myoglobin with nitric oxide. The preservative, sodium nitrite, has been found to be the usual source of the nitric oxide. When in solution, the nitrite ion exists in equilibrium with undissociated nitrous acid. The nitrous acid then decomposes in slightly acid conditions to give nitric oxide (Pegg and Shahidi, 2000).

The nitrite also serves as an oxidizing agent which rapidly converts myoglobin to metmyoglobin, which is then reduced to nitrosyl metmyoglobin. Ranken (2000) added that the conversion of myoglobin to the nitrosyl form had been found to be incomplete, inconsistent and may vary between about 35% and 75%. The percentage is dependent on the input nitrite of 100-150ppm in different samples of meat. During heating, nitrosyl myoglobin is denatured to pink nitrosyl myochromogen, also known as the Cooked Cured Meat Pigment (CCMP) (Pegg and Shahidi, 2000). Pegg and Shahidi,

(2000) further explained that the colour intensity of the cured meat is directly proportional to the concentration of nitric-oxide stabilised myoglobin in the muscle and not the nitrite level.

During meat curing, some additives such as ascorbic acid, ascorbate and erythorbate play a role by influencing the meat colour by serving as catalyst. They do this by increasing the conversion of nitrite to nitric oxide which in turns speed up the reaction of nitric oxide with myoglobin (Sebranek and Bacus, 2007). Ascorbate not only improves the efficiency of curing but also its reaction helps save approximately onethird of the nitrite. It also helps to remove traces of oxygen, which prevents meat colour development during curing (Ranken, 2000).

Studies have shown that residual nitrite levels of 45-119ppm are enough to produce the desirable cured meat colour (Sebranek and Bacus, 2007). Tichivangana et al (1984) added that levels down to 10mg/kg of nitrite in lean meat will produce desirable colour and flavor.

The nitrite level required for colour fixation is dependent on the concentration of myoglobin present in the meat tissue. Myoglobin's concentration in the tissue is dependent on certain factors such as species, sex, age, muscle and nutrition. For meat colour to be stable, the residual nitrite plays a role by acting as a reservoir for nitric oxide for continued stabilization of colour pigment (Dryden and Birdsall, 1980; Walsh et al, 1998).

2.8 Cured meat flavour

The production of cured meat flavour may be due to the effect of nitrite on lipid oxidation. Studies have shown that nitrite delays lipid oxidation in meat products there by delaying rancidity which influences flavour (Gray and Pearson, 1984). The production has been found to be similar to that which produces colour, by reducing iron in the haem compound to a form which does not promote oxidation (Pierson and

Smoot, 1982).

Comparing nitrite with other antioxidants, it was found to show a better flavour outcome since the flavour development from the others was not similar to that of the nitrite (Sebranek and Bacus, 2007). Further studies have shown that nitrite levels down to 50ppm can have an antioxidant effect which reduces thiobarbituric acid (a measure of rancidity) values by up to 64% for beef, chicken and pork (Sebranek and Bacus, 2007). A study by Feiner (2007) has shown that the flavour development is as a result of nitrite components reacting with sulphuric material which is present in meat muscle.

2.9 Possible formation of nitrosamines

In spite of nitrate and nitrite being the major preservatives used in cured meat production, they are also naturally occurring ions which are highly distributed in our diets such as in vegetables and fruits. Ingested nitrate into the body comes out in the saliva and also bacteria present in the mouth reduced it to nitrite. A study by IARC (1987) shows that nitrite ions can react readily with nitrosatable compounds in the stomach, especially secondary amines and alkyl amides, to generate N-nitroso compounds (human carcinogens) under acid conditions. Further epidemiological studies, showed that people with a high intake of nitrate or nitrite and a low intake of vitamin C were at a high risk when assessed with studies without this information since these could result in endogenous formation of N-nitroso compounds (IARC, 2010).

In the 1960s and 1970s, the use of nitrate and nitrite became a major concern due to the formation of nitrosamines which are carcinogenic and as such research into alternative preservatives began to also reduce the nitrite level we ingest into our bodies. Efforts made by researchers on the study to reduce nitrite level were proved by Cassens (1997) where

nitrite usage was found to be decreasing in the USA. The study concluded that the residual nitrite content of cured meat at retail was 10ppm, representing an 80% reduction since the 1970s.

Studies from Shahidi and Pegg (1993) showed that nitrosamines were found to be present after cooking certain cured meat products at high temperatures. Carcinogenic nitrosamines are formed when amines that occur naturally in food react with sodium nitrite found in cured meat products (Najm and Trussell, 2001) and N-nitrosopyrrolidine (Bills et al, 1973) form carbocations that react with biological nucleophiles (such as DNA or an enzyme) in the cell.

2.10 Antimicrobial property of nitrite

Nitrite not only improves the meat colour and flavour but also has a microbiological effect on the meat product. It is used to achieve safety with respect to food pathogens by inhibiting growth of spore forming bacteria such as *Clostridium botulinum*. Benedict (1980)

Benedict (1980) explained the mode of action of nitrite on its microbiological effect on pathogens. In spite of the fact that the action of nitrite is not well known, it has been considered to have many possible target sites of action in the microbial cell. Benedict (1980) further explained this mode of action by reviewing the biochemical basis for nitrite inhibition of C. botulinum in cured meat. There are five main primary modes of action (Singhal and Kulkarni, 2000; Surekha and Reddy, 2000) which are outlined below.

i. inactivation of key enzymes to inhibit respiration,

ii. release of nitrous acid and nitric oxides . iii. reduction in the levels of intracellular ATP.

iv. reduction in the efficiency of the active transport systems by blocking important enzyme pathways.

v. formation of S-nitroso compounds by reaction of nitrite with haem proteins.

2.11 Nitrite loss

Most of the nitrite added to meat during the manufacturing stage for curing meat products is depleted in the process as a result of a series of nitrogen oxide reactions during processing and storage. Between only about 10% and 20% of the added nitrite may remain after the manufacturing process. This residual nitrite also then further declines slowly during the storage of the meat products (Sindelar and Milkowski, 2012)

Some factors such as pH, heat treatment, and storage temperature and ascorbate presence tend to affect the level of residual nitrite in food products. After 25 days storage of cured meat product, Sofos et al (1980) report that the residual level of nitrite goes down between 1 and 3ppm irrespective of whether 40, 80 or 120ppm was added to the original meat product. A study by EFSA (2003) stated that, to control *C. botulinum*, the level of residual nitrite is very important not forgetting the ingoing nitrite amount in the meat product. For example, (*C. botulinum*) spores have been found to germinate once the residual nitrite levels fell below 10mg/kg (Christiansen, 1980). And further studies show that they grow rapidly with reducing residual nitrite when the starting nitrite falls.

2.12 Nitrite effect on food pathogens

Nitrite as a preservative also serves the purpose of inhibiting the growth and multiplication of dangerous and harmful microbes capable of causing meat spoilage and poisoning. The effect of this activity of nitrite on meat products also extends the self-life of the meat products and provides safety for consumers. (Russell and Gould, 2003)

2.12.1 Clostridium botulinum

Rhodehamel *et al* (1992) stated that nitrite levels of 120 to 200 ppm (mg/kg) are enough to protect against C. botulinum growth and prevent toxin production. Further study by Roberts *et al,* (1981) suggested that with nitrite input of 100mg/kg, the production of toxin was found to be 54% but when increased to 300mg/kg, toxin production was only 1% under acidic condition (pH of 6). Pierson and Smoot (1982) added that to achieve food safety, the nitrite level needed to protect the meat product from bacterial growth, for instance, and *C. botulinum* has been established.

2.12.2 Clostridium perfringens

Gibson and Roberts (1986a) studied the combined effects of salt and nitrite on *Clostridium perfringens* using different temperatures and pH values. The outcome of their work was that at storage temperatures of 20 to 35ºC, growth of *C. perfringens* was inhibited when up to 200mg/kg of curing salts were used under acidic conditions (pH of 6.2 or below). It is important to note that, after the addition of the curing salts, 3% of salt was also added. They concluded that a temperature below 20ºC permits the slow growth of *C. perfringens* without the addition of nitrite or salt.

2.12.3 Listeria monocytogenes

Nitrite use in cured meat products has been found to inhibit *Listeria monocytogenes* (Buchanan *et al*, 1989). And just as *C. perfringens*, the conditions needed to induce inhibitory effect on L. monocytogenes were temperature and pH. Further studies showed that under acidic conditions (pH of 5.3 or below), no growth of *L. monocytogenes* was detected within 21 days when 50mg/kg of nitrite was used (McClure *et al*, 1991). It was also found that the condition of action of the nitrite is very important since there was little nitrite effect on the organism at pH 6.0 and above even when 400mg/kg of the nitrite was used at a temperature of 10ºC.

2.12.4 Salmonella

With respect to Salmonella, Gibson and Roberts (1986b) observed that inhibition only occurs under extreme conditions of pH 5.6, 10ºC and 400 ppm nitrite. At temperatures between 10ºC and 35ºC, the bacterial growth was not inhibited by most combinations of salt (1 – 6% w/v), pH (5.6, 6.2 and 6.8) and sodium nitrite (0-400 ppm) when tested. A study by Rice and Pierson (1982) showed that Salmonella growth was inhibited in frankfurters containing 156 ppm nitrite when stored at 15ºC but not at 27ºC. They also noted that 50ppm nitrite did not inhibit growth at either temperature.

2.12.5 Escherichia coli

Buchanan and Bagi (1994) further proved that microbial action can be stopped by sodium nitrite under acidic conditions (pH values <5.5) and low temperature when tested on *E. coli*. Their study showed that at pH 5.5 and 28ºC there was an extension of lag time and decrease in growth rate at 200 ppm nitrite. They further concluded that at pH 4.5 a level of 200 ppm nitrite was bactericidal.

2.12.6 Staphylococcus aureus

Studies showed that *Staphylococcus aureus* may be tolerant to the antimicrobial effect of nitrite and that for bacteriostatic effect to occur pH and oxygen availability are needed. The tolerance action of this organism was experienced in the study by Buchanan *et al*. (1993) which showed that an increase in salt level of up to 200mg/kg nitrite, bacterial growth still occurred. Smith and Palumbo (1980) also found that *S. aureus* growth was inhibited by nitrite.

2.13 Other additives with microbiological effect

2.13.1 Sorbate

Sorbate has been shown to have antimicrobial action in spite of its anti-fungal action. Microorganisms such as *C. botulinum*, Salmonella and *S. aureus* have been found to be inhibited by sorbate. For instance, studies by Sofos et al (1979) and Tompkin et al (1974) showed that sorbate is effective in inhibiting sporulation of *C. botulinum,* provided the pH is low enough and that the sorbate is in high concentration. 2000ppm potassium sorbate was found to have an inhibitory action on non-proteolytic *C. botulinum* in broth studies where 2% salt was present and the pH was 5.5 at 30ºC

(Jones and Betts, 2009).

Rice and Pierson (1982) demonstrated that sorbate at a level of 2,600 or 3,900 ppm was effective to inhibit Salmonella growth in frankfurters either alone or in combination with 50 or 156ppm nitrite at the temperature of 15ºC or 27ºC for up to 21 days. Smith and Palumbo (1980) added that S. aureus growth was inhibited when 2,500 ppm of sorbate was added in a model sausage system at 35ºC for 3 days under anaerobic conditions but 5,000 ppm was required to inhibit growth under aerobic conditions.

2.13.2 Benzoate

A solution containing benzoate at a concentration of 25% w/v has been found to prevent *Listeria monocytogenes'* growth on the surface of frankfurters (Islam et al 2002a) and sliced meats (Islam et al., 2002b). Zhao et al (1993) demonstrated that 0.1% solution of benzoate inhibited the growth of Escherichia coli in apple juice. With *C. botulinum*, Jones and Betts (2009) showed that 2,000 ppm sodium benzoate had an inhibitory action in broth studies where no salt was present; the pH was 7.0 at storage of 8ºC.

2.13.3 Lactate

Lactates tend to be added to foods for their taste, buffering ability and humectant properties (Luck and Jaeger, 1997; Davidson et al 2005). Lactates have been found to have preservative action in spite of their buffering ability and humectant properties (Davidson et al, 2005). Under acidic condition (pH below 5.2), Notermans and Dufrenne (1981) demonstrated that formation of toxins by proteolytic *C. botulinum* in a meat slurry is reduced. Further studies on *C. botulinum* showed that lactate mediated inhibition of toxin formation at 11^{oC} lower temperatures, and that the effect was not due to lowering the water activity of the broth system used (Houtsma et al, 1994).

2.14 Effect of cooking on nitrite

Heat application to cured meat products shows some effect on nitrite. Studies show that overcooking cured meat products may result in the formation of genotoxic compounds during digestion and it needs to be avoided (Van Hecke et al., 2015). Deep frying or pan-frying cooking methods have been found to increase the concentrations of volatile N-nitrosamines but boiling or microwave treatment does not show any significant difference from the raw product (Li and Xu, 2012). This proves that boiling and microwave treatments were suitable cooking methods for cured meat products.

Furthermore, a study by Herrman *et al* (2015) showed that frying and baking of processed meat tends to increase the levels of N-nitrosopiperidine but decreases levels of non-volatile nitrosamines such as N-nitroso-thiazolidine-4-carboxylic acid and Nnitroso-2-methyl-thiazolidine4-carboxylic acid. They further explained that depending on the type of meat product or heat treatment (cooking method), varying impacts of Nnitosoproline, N-nitrosodimethylamine, N-nitrosopyrollidine, N-nitrosodimethil-amine and nitrosodiethylamine and N-nitrosomethylaniline are observed.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Eighteen (18) pieces of six (6) different brands of sausages were purchased from supermarket, mini market, open markets and cold stores in the Ashaiman community in the Tema district of the Greater Accra region of Ghana for the entire analysis and transported in an ice chest stored in plastic bags and then kept in a refrigerator prior to analysis.

3.2 Reagents

A pellet of sodium hydroxide was added to prevent liberation of nitrous acid and 1.0 ml of chloroform was added to inhibit bacterial growth (ENSAFI et al., 2004). Each working

standard solution was freshly prepared by diluting the stock solution with distilled water. The chemicals used were pure sodium nitrite, Sulphanilamide, N-1Naphthylethylene diamine dihydrochloride and sodium nitrite used for the analyses was purchased from BDH chemical Ltd - England and stored in a cool dry place. All chemicals used were of analytical grade and deionized water was used throughout the experiments.

3.3 Food sampling and preparation

A total of 18 samples of six (6) different brands of sausages were randomly purchased irrespective of the size in supermarkets, minimarkets, and cold stores and open markets in the Ashaiman community of Tema District of Greater Accra region of Ghana. The samples were checked for date of expiration to reduce any chances of working with expired or spoiled sausage, all samples bought had almost 2 months shelf life before expiration. The samples were checked for their nitrite levels in the sausages in its uncooked (fresh) state and compared to the nitrites levels after the sausages had been subjected to cooking methods by three (3) different selected cooking methods; pan frying, boiling and grilling.

Five grams of each treated sample was transferred into a 50 ml beaker and 40ml of water heated to 80^0C added. It was mixed thoroughly with a glass rod taking care to break all lumps and transferred to a 500ml 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 the mashed samples to increase the volume to about 300ml and transferred to a steam bath and allowed to stand for 2 hrs. with occasional shaking. After cooling to room temperature, it was diluted to volume with pure water, remixes and filtered. The process was repeated for each brand under each cooking state,

each cooking method with the different brand was done for a day and the collected filtrate stored in a refrigerator till the final analysis of the nitrite determination was done.

3.4 Cooking methods

Six different commercially available sausages were used in this study and were cooked using three (3) different methods of cooking in homes and outside homes, namely, boiling, pan-frying and oven grilling. One kilogram of sausage per brand was taken from the already acquired products and used for analysis. The samples were divided into four (4) portions of equal quantity, one portion for each cooking method and the last portion serving as the control for the analysis.

Boiling was done by the addition of water to the sausages in a sauce pan and cooked till ready for consumption. The sausage was then drained of all the water and made to cool. Pan-frying was also done to the sausage as it was cooked in frytol cooking oil till ready for consumption. The cooked sausage was removed and drained all the oil and made to cool. The third portion of the samples was grilled in an oven till ready for consumption and made to cool. The same procedure was applied for the other 5 brands of the sausage for completion for the analysis. Throughout the whole cooking process, moderate heat was applied to obtain optimized cooking throughout all the three cooking methods. The raw (uncooked) sausage serving as the control and all the cooked sausages were homogenized.

3.5 Nitrite sample extraction

Both the raw and cooked sausage samples were mashed and homogenized with the help of a mortar and pestle. Ten (10 g) grams of the homogenized samples was weighed and transferred into a 500mL volumetric flask from a beaker, followed by the addition of 40

mL of 70ºC heated pure water. The mixture was then mixed thoroughly by shaking and also using of a glass rod to break any possible lump present. The volume was then increased with the addition of extra water to a level of 300 mL. Volumetric flask with the mixture was then placed in a water bath to cool and allowed to stand for about 1hr at room temperature. The supernatant liquid was carefully obtained by filtration to get a fine clear extract for the real analysis.

3.6 Preparation of standard nitrite solution

1.00 gram of pure dry nitrite was weighed and dissolved in distilled water and later diluted to one liter (1L) volumetric flask. Then 100 ml of the stock solution was pipetted and placed in the volumetric flask. The working solution is prepared by diluting in appropriation to desired amount. The working solution was equal to 1 ppm.

3.7 Preparation of the standard calibration curve

A quantity of 10, 20, 30 and 40 ml of nitrite working solution were taken into a 50 ml volumetric flask. A 2.5 ml of the chemical Sulphanilamide reagent (sulfa) was added to the measured quantities of working nitrite solution in the flask and made to stand for about 5 minutes, then, same quantity of 2.5 ml of naphthylethylenediamine hydrochloride (NED) reagent was also added and made to stand for about 5 min. The mixture was then shaken to mix well and then made to stand for about 15 min.

Some portion of the mixture was then taken and transferred into a photometric cell and its absorbance was then checked and the readings taken at 538 nm against a blank solution of 45 ml of distilled water in addition to 2.5 ml of sulfa and 2.5 ml of NED reagents.

3.8 Nitrite determination

A 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 $NaNO_2$ were prepared from a stock solution of 1mg/L and measured using Shimadzu UV-Vis (Model =UVmini-1240). 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 was determined. All the experiments were performed in triplicate and the results were expressed by their means values.

3.9 Statistical analysis

The data given from the nitrite in the sausage in each product brand were the mean value of the three (3) cooking methods applied. The collected data were entered in SPSS-23 software and inter-group in $\alpha = 0.05$ were analyzed by ANOVA statistical tests and presented by descriptive statistics. All data obtained was analyzed using oneway Analysis of Variance (ANOVA, SAS, 1985) to determine whether there was a significant difference in the nitrite concentration of the six (6) commonly consumed brands of sausage cooked by three different methods. A two-way ANOVA was also used to determine the interaction effect of sausage brands and cooking methods on nitrite levels using Tukey- Pairwise comparisons between the means. Significance was determined at the 95% confidence interval by ($\alpha < 0.05$)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Nitrite levels in sausage samples

Table 1 shows the uncooked (raw) mean nitrite values of the six selected brands of sausage compared to residual nitrite values obtained from three (3) cooking methods applied to the same raw sausages

Table 1 Mean nitrite content (ppm) of uncooked and cooked sausage brands.

Sausage Brands	Uncooked	Boiled	Fried	Grilled
German Veinna.	164 ± 0.76	78 ± 0.17	81 ± 0.21	80 ± 0.29
Nempa	248 ± 0.46	218 ± 0.29	269 ± 0.20	387 ± 0.31
Adom	108 ± 0.28	104 ± 0.23	217 ± 0.47	198 ± 0.17
Bino	187 ± 0.25	154 ± 0.29	225 ± 0.19	150 ±0.05
Frankfuter	188 ± 0.38	54 ± 0.24	45 ± 0.12	47 ± 0.17
Imperial	347 ± 0.07	190 ± 0.36	218 ±0.14	205 ±0.41

The residual nitrite concentration of the brand German vienna sausage in the un-cooked (raw state) 164 ppm was not within the allowable residual nitrite level for sausage therefore, exceeded the set limit for residual nitrite in sausage, which is 125ppm (Ghana Standards Authority, 2011). In terms of the cooking methods employed and its effect on the residual nitrite level of the sausage brand; all three cooking methods had a decreasing effect on the residual nitrite level from the uncooked nitrite concentration. Boiling had the better reduction effect (78 ppm) compared to grilling (80 ppm) and pan frying (81 ppm) respectively.

The residual nitrite concentration of the brand Nempa sausage in the un-cooked form as 248 ppm was much higher than the maximum acceptable limit (125ppm) for residual

nitrite in cured meat product including sausages for consumers. Comparing the levels of nitrite from the cooking methods used to the raw nitrite level, boiling (218 ppm) was the only cooking method that had a reduction effect on the nitrite level in raw sausage. Pan frying (269 ppm) and grilling (387 ppm) all showed an increase from the initial nitrite level in the raw sausage. Among the cooking methods, boiling (218ppm) had the lowest and grilling (387ppm) had the highest nitrite level with pan frying (260ppm) being the intermediate.

The mean nitrite level in the uncooked Adom sausage nitrite concentration (108ppm) was within the recommended acceptable maximum limit for cured meat product including sausage. Comparing the levels of nitrite from the cooking methods used to the raw nitrite level, boiling (104ppm) was within the acceptable limit and the only method which had a reduction effect on the nitrite level in raw sausage. Pan frying (217ppm) and grilling (198ppm) all showed an increase from the initial nitrite level in the raw sausage. Among the cooking methods, the mean value for boiling (104ppm) had the lowest nitrite level, followed by grilling (198ppm) and pan frying having the highest nitrite level with (217ppm).

The nitrite level in the uncooked Bino sausage brand (187 ppm) was higher than the recommended acceptable maximum limit. Comparing the levels of nitrite from the cooking methods used to the raw nitrite level, boiling (154 ppm) had a reduction effect on the nitrite level in raw sausage but was not within the acceptable limit, Pan frying (255ppm) had an increasing effect on the raw nitrite level which was way above the acceptable limit with grilling (150ppm) which showed a reduction from the initial nitrite level in the raw sausage. Among the cooking methods, the mean value for Bino sausage,

grilling (150 ppm) had lowest nitrite level, followed closely by boiling (154 ppm) and pan frying having the highest nitrite level with (217 ppm) which is an increase from the raw nitrite level (187 ppm).

The sausage brand of Frankfuter has a residual nitrite concentration level of 188 ppm for its un-cooked (raw) form, which is not within the maximum recommended level of 125 ppm. Comparing the levels of nitrite from the cooking methods to the raw nitrite level from the table above, boiling (54ppm), and pan frying (45 ppm) and grilling (ppm) all had a reduction effect on the nitrite level from raw sausage and also well within the recommended acceptable limit for nitrite in cured meat including sausages. Among the cooking methods, the mean value for Bino sausage, pan frying (45 ppm) showed the lowest nitrite level, followed closely by grilling (47 ppm) and boiling, having the highest nitrite level with (54 ppm).

The raw Imperial sausage had nitrite concentration of 347ppm. However, boiling (190 ppm), grilling (205 ppm) and pan frying (218 ppm) all had a reduction effect on the nitrite level from raw sausage but not within the recommended acceptable. Among cooking methods, the mean value for imperial sausage, boiling (190ppm) had lowest the nitrite level, followed by grilling (205ppm) and pan frying having the highest nitrite level with (218ppm).

4.2 Effects of different cooking methods on nitrite level

As shown in table 1 above, the mean nitrite values of all the cooking methods; boiling (132.89ppm), pan frying (177.93ppm) and grilling (181.57ppm) including the raw (206.97ppm) which was the control all had high values above the recommended acceptable limits (125ppm) by the Ghana standard authority (2011). In a separate study

carried out in Denmark (Leth *et al.,* 2008), the results showed that most processed meat including sausage products studied, was below Denmark's recommended limit for nitrite in cured meat product (60ppm for most product and 150ppm for certain special products) set limit by Danish Veterinary and Food Administration.

The effect of cooking methods on the content of residual nitrite in sausage is displayed in table.1 The results, expressed in mean values showed that all three cooking methods did reduce the residual nitrite, however, boiling treatment with raw sausage showed a strong correlation at 67% (Appendix 2, Table.2) and showing much significance at $p>0.05$ (Appendix 2, Table 3). This could be explained by the solvent nature of water compared to oil and grilling. The effect of boiling on the residual nitrite level of the sausage did agreed with the similar research by (Ya *et al*., 2016) did conclude that cooking by boiling reduce residual nitrite in sausage. Also, in another study, carried out by Li et al (2012), on the influence of cooking methods on the concentration of volatile N-nitrosamines, in dry- cured sausages, the study concluded that residual nitrite was significantly reduced by cooking process.

Table 2: Mean residual nitrite levels in raw and cooked sausages

Cooking Methods	Residual Nitrites Levels	
	Mean (ppm)	Standard Error
Raw sausage	206.97 [a]	33.57
Boiled sausage	132.90[b]	26.60
Fried sausage	177.93[a]	49.16
Grilled sausage	181.57[a]	38.54

*Values with the same letter denote no significant difference ($p > 0.05$).

The residual nitrite concentration level in the raw sausages had the highest mean value of 206.9±33.6 ppm whereas the boiled sausage samples had the least mean value of 132.9±26.6 ppm was significant at ($p > 0.05$) with the letter (b) in the table above. Fried and grilled with nitrite mean values of 177.9±49.2 ppm and 181.6±38.5 ppm, respectively were not statistically significant at ($p > 0.05$) from the table above having common letter (a). Among the cooking methods employed, cooking by boiling significantly reduced the nitrite level from the uncooked nitrite level.

4.3 Comparison of nitrite levels in sausage brand by cooking effect

The effects of brands by cooking method interaction on the residual nitrite levels were assessed using ANOVA (two-way) at the α-level of 0.05. The results indicated that the interaction of brands and cooking methods is significant ($p = 0.000$) and therefore a comparison analysis was required to further explain which levels of the interactions were significant. A Tukey pairwise comparison of the nitrite levels of brands by cooking method shows that fried Nempa had significantly higher nitrite concentrations than all the cooked forms of all the other brands except for raw Imperial, with which it was similar. On the other hand, Grilled Frankfurter had significantly lower nitrite concentrations than fried Nempa, raw Imperial, grilled Nempa, grilled Imperial, raw Nempa, grilled Bino, boiled Nempa, grilled Adom, fried Imperial, fried Adom, boiled Imperial, raw Frankfurter, raw Bino and raw German sausages whereas it had similar nitrite concentrations with boiled Bino, fried Bino, raw Adom, boiled Adom, grilled German, fried German, boiled German, boiled Frankfurter and fried Frankfurter.

Table 3. Comparison of nitrite levels in different sausage brands by cooking effect.

BRANDS	COOKING METHODS (ppm)			
	RAW	BOILING	FRYING	GRILLING
GV	164.31[a]	77.76[c]	79.79g[b]	81.43[b]
NP	248.27[c]	218.35[c]	387.63[b]	269.27[a]
AD	107.59[c]	103.77[d]	197.79[b]	217.05[a]
BN	187.12[b]	153.48[c]	149.82[a]	225.10[d]
FKT	187.58[a]	53.533[b]	47.28[d]	44.68[c]
IMP	347.10[a]	192.48[a]	205.51[a]	251.89[a]

different letters in a row indicates significantly different

The results of residual nitrite levels in the brands by cooking effect, shows which cooking method is best for a particular brand. The aim is to reduce the nitrite levels within each sausage brand within the acceptable limits safe for human consumption. From the results, it was observed in row one and five that, the German and Frankfurter brands which had 188ppm and 164ppm, respectively as the mean nitrite value in the uncooked sausage, were reduced at a significance of ($p < 0.05$) nitrite levels after all the cooking methods was carried out on them, this makes them an ideal sausage to be used by all cooking methods.

However, brands Nempa and Adom, which had low residual nitrite level from the raw form showed an increase in its nitrites concentration when applied by the three cooking methods. Comparing the nitrite content after the cooking methods were done, especially in fried Nempa and grilled Adom sausages, there was a sharp contradiction to the earlier

two brands discussed. Additionally, these two brands were the only local brands in the brands surveyed indicating that the local sausage production companies need to be monitored strictly to abide by the internationally accepted standards for nitrite concentration and also, try to incorporate measures that can stabilize the nitrite levels in their products. Also, from the table above, imperial sausage was not significant throughout the cooking method.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Nitrates and nitrites are two most important additives in meat industry because of their beneficial effect on the quality and microbiological safety in meat products. On the other hand, major concern of nitrates and nitrites in meat products is related to the potential of nitrites to form carcinogenic N-nitroso compounds which is of great health concern for consumers.

Based on the findings of the study, nitrite levels among the six (6) brands of sausage in the uncooked form were higher than the set limit of (125 ppm) by GSA for sausage meat product in Ghana except for Adom sausage brand (108 ppm).

Sausage manufactures and importers do not adhere to the required quantity of nitrate and nitrite as usage of nitrate and nitrite in sausages found on the market of Ashiaman, Ghana were in higher levels.. In the case of the different cooking methods and its effect on the nitrite concentration of the six (6) sausage brands. Cooking by boiling significantly reduce the nitrite content at ($p < 0.05$). Cooking by grilling and frying does not significantly decrease nor increase the nitrite concentration of the commercially available sausages in Ashaiman constituency of Tema, Ghana.

5.2 Recommendations

The study conducted focused only on sausage and therefore recommend that further evaluation must be focused on all other processed meat product, both foreign and local. It is also recommended that further studies be done on the same products which will focus on the levels of nitrosamines and other nitroso compounds to evaluate the carcinogenicity

of cured sausage and other cured meat products. The regulatory bodies such as the GFDA and the GSA must ensure proper checks are done on these product found on the market to instill consumer confidence and also help regulate the use of this preservative during production especially local production companies. Finally, from the results obtained in this study, it is clear that sausages on the market have high residual nitrite content, therefore, it is important for FDA to do post product analysis check to confirm the right amount of the nitrite were used in the finished product on the market.

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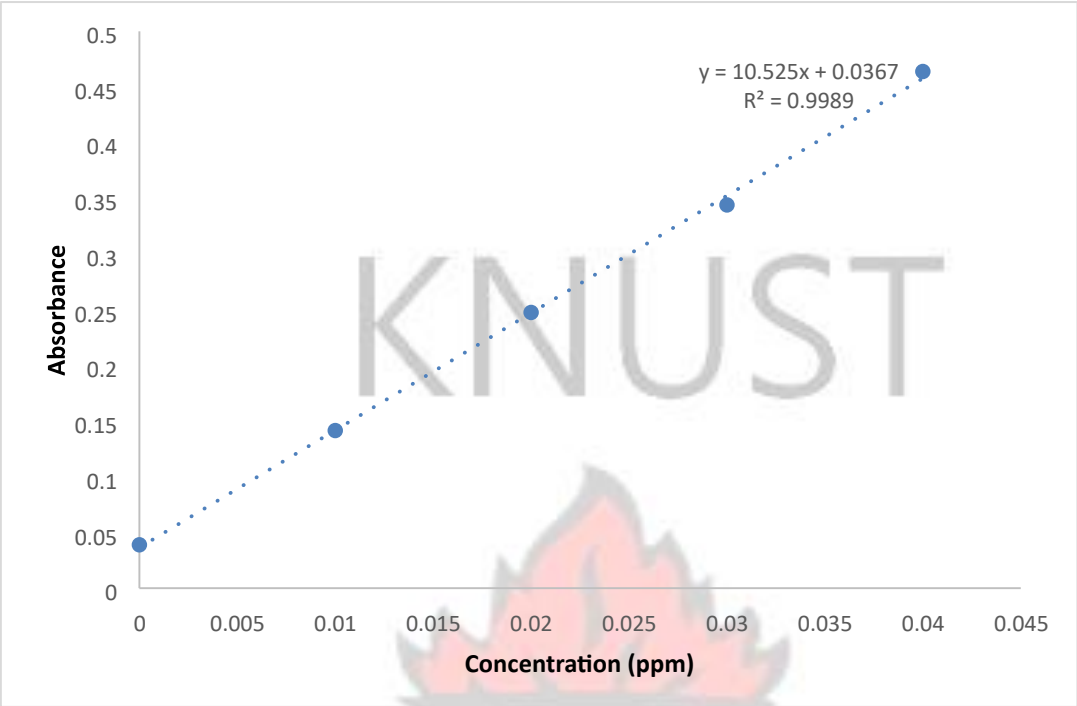
APPENDIX

Appendix 1: Nitrite standard calibration curve for determining nitrite concentration in sampled processed meat products

Volume of standard solution (ml)	Absorbance	Concentration (ppm)
BLANK	0.000	0.00
10	0.142	0.01
20	0.248	0.02
30	0.344	0.03
40	0.464	0.04



Appendix 2: ANOVA results showing significance of treatment of raw sausage brands.

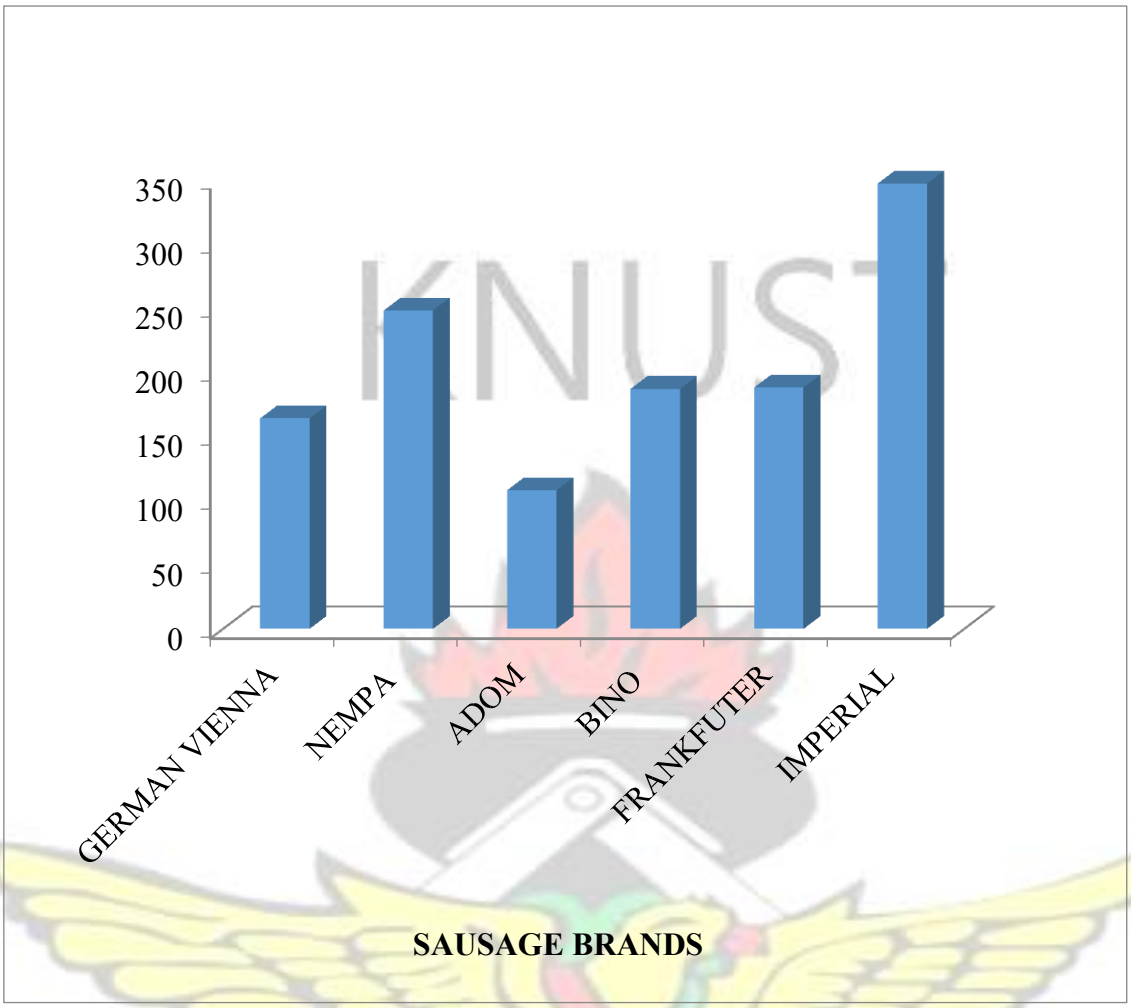
	Sum of Squares	df	Mean Square	F	Sig
Treatment for Raw sausage	101439.684	5	20287.937	39496.106	.000
Between Groups	6.164	12	.514		
Within Groups	101445.848	17			
Total					

Treatment for Boiled sausage	63685.080	5	12737.016	57840.147	.000
Between Groups	2.643	12	.220		
Within Groups	63687.722	17			
Total					
Treatment for Grilled sausage	133673.136	5	26734.627	5.353	.008
Between Groups	59937.201	12	4994.767		
Within Groups	193610.337	17			
Total					
Treatment for Fried sausage	217525.310	5	43505.062	388726.563	.000
Between Groups	1.343	12	.112		
Within Groups	217526.653	17			
Total					

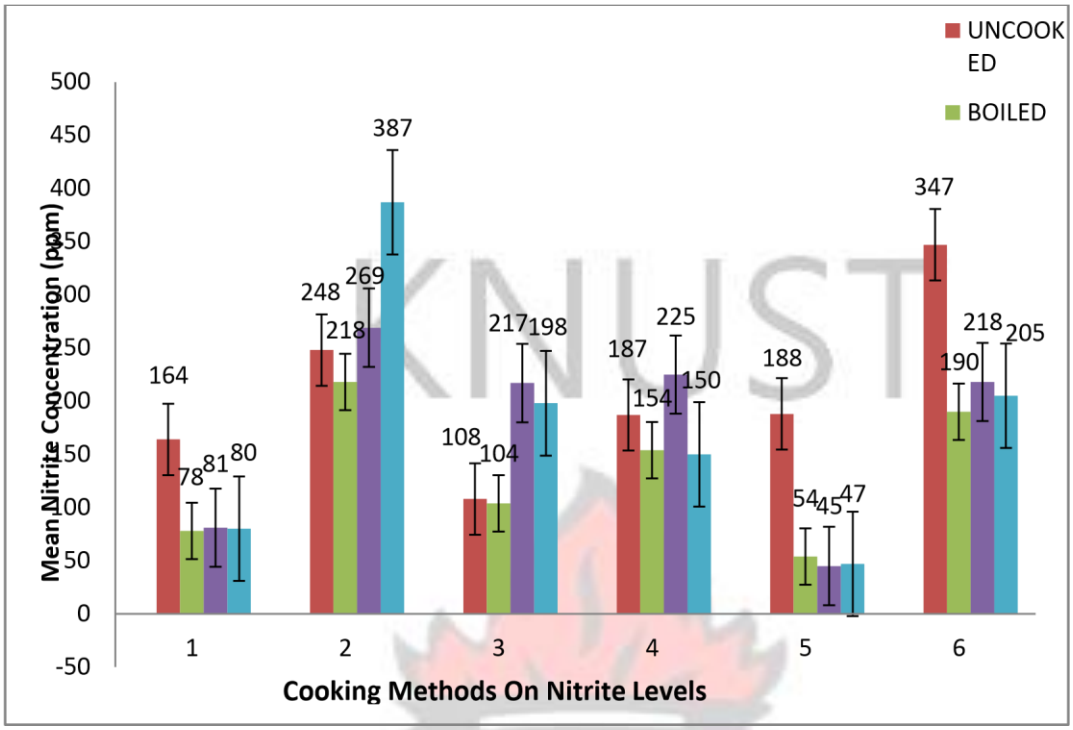
	N	Correlation	Sig

Pair 1 treatment for Raw sausage & treatment for Boiled sausage	18	.671	.002
Pair 1 treatment for Raw sausage & treatment for Grilled sausage	18	.343	.164
Pair 1 treatment for Raw sausage & treatment for Fried sausage	18	.360	.142

Appendix 3: Nitrite levels of raw sausage (ppm)



Appendix 4: Effect of cooking methods on the nitrite levels of six brands of sausage.



Appendix 5

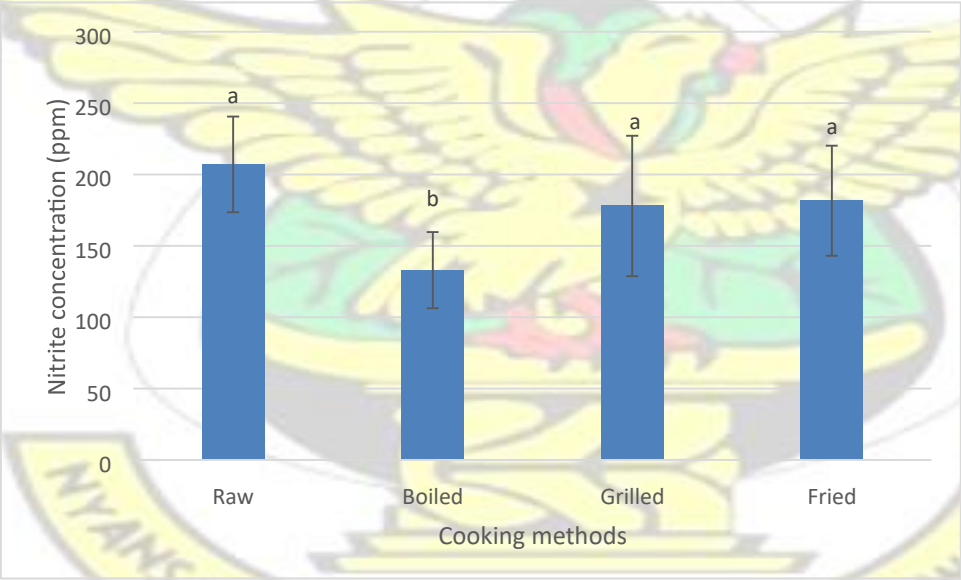


Figure 5: Mean nitrite concentrations in cooking methods with standard error bars, different alphabets denote significant differences between means.

Appendix 6

Tukey Pairwise Comparisons: Response = NITRITE LEVELS, Term =

COOKING METHOD

Grouping Information Using the Tukey Method and 95% Confidence

COOKING METHOD	N	Mean	Grouping
RAW	18	206.993	A
GRILLING	18	181.569	A
FRYING	18	177.927	A
BOILING	18	132.897	B

Means that do not share a letter are significantly different.

Tukey Simultaneous 95% CIs

Tukey Pairwise Comparisons: Response = NITRITE LEVELS, Term = BRANDSS*COOKING METHOD

Grouping Information Using the Tukey Method and 95% Confidence

BRANDSS*COOKING METHOD	N	Mean	Grouping
NP FRYING	3	387.363	A
IMP RAW	3	347.103	A B
NP GRILLING	3	269.267	B C
IMP GRILLING	3	251.890	B C D
NP RAW	3	248.247	B C D
BN GRILLING	3	225.103	C D
NP BOILING	3	218.350	C D E
AD GRILLING	3	217.047	C D E

IMP FRYING	3	205.513	C D E F
AD FRYING	3	197.790	C D E F
IMP BOILING	3	190.483	C D E F G
FKT RAW	3	187.583	C D E F G H
BN RAW	3	187.123	C D E F G H
GV RAW	3	164.307	C D E F G H I
BN BOILING	3	153.480	D E F G H I J
BN FRYING	3	149.823	D E F G H I J
AD RAW	3	107.593	E F G H I J
AD BOILING	3	103.773	F G H I J
GV GRILLING	3	81.433	G H I J
GV FRYING	3	79.793	G H I J
GV BOILING	3	77.763	H I J
FKT BOILING	3	53.533	I J
FKT FRYING	3	47.277	J
FKT GRILLING	3	44.677	J

Means that do not share a letter are significantly different.

* NOTE * Cannot draw the interval plot for the Tukey procedure. Interval plots for comparisons are illegible with more than 45 intervals.