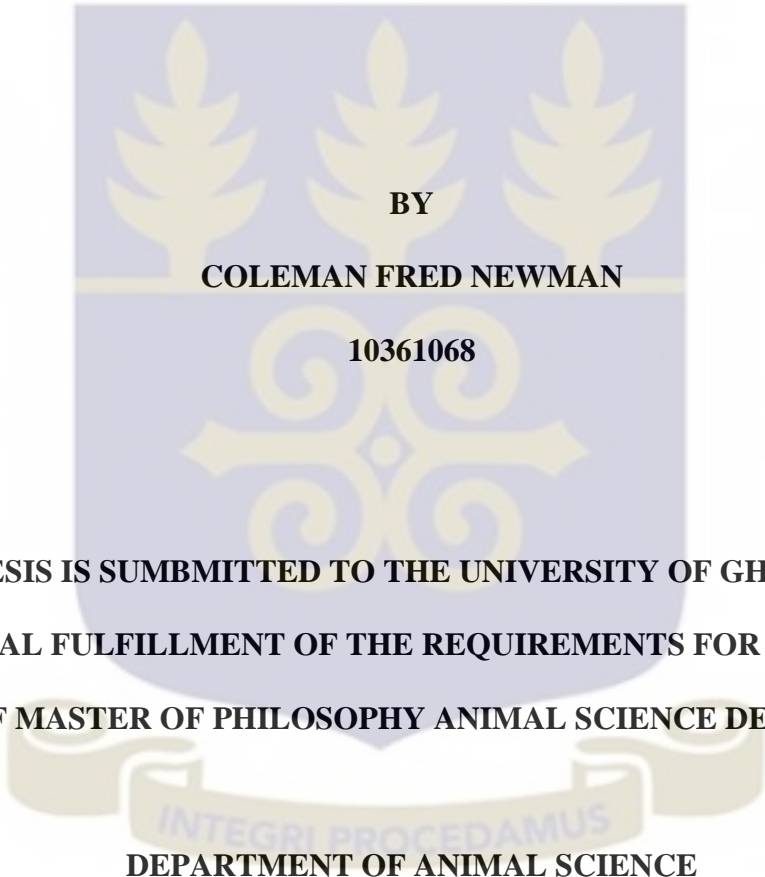


**UNIVERSITY OF GHANA**

**EVALUATION OF SELECTED LOCAL SPICES ON SENSORY AND  
MICROBIAL CHARACTERISTICS OF FRESH PORK SAUSAGE**



**BY**  
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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA LEGON,  
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**DEPARTMENT OF ANIMAL SCIENCE  
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UNIVERSITY OF GHANA**

**JULY, 2015**

**DECLARATION**

I hereby declare that this thesis which is submitted to the Department of Animal Science, College of Basic and Applied Sciences, University of Ghana, for the award of Master of Philosophy in Animal Science degree is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere. All assistance towards the production of this work and all the references contained herein have been duly credited.

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## DEDICATION

I dedicate this work to my late mother; Rose Esinu Betsoe, my foster parents; Mr. & Mrs. Beresford Ruth, my wife; Mildred Anthonio Coleman and my son Nii Noi Ridley Eyram Coleman.



## ABSTRACT

*Xylopiya aethiopyca* (African pepper) and *Monodora myristica* (African nutmeg) are used as spices in Ghanaian local dishes and as traditional medicine. The objective of this study was to substitute two spices in “normal” fresh pork sausage formulation with selected local spices and evaluate their effect on sensory and microbiological characteristics of the product. A 4x4 factorial design was used with 4 spice treatments (Control – *Syzygium Gaertner* (clove), *Allium cepa* (onion), *Piper nigrum* (white pepper) and *Myristica fragrans* (nutmeg); African pepper (AP) substituted for white pepper; African nutmeg (AN) substituted for nut meg (*Myristica fragrans*); and combination of AP and AN (AP\*AN) at 4 inclusion levels (0%, 0.05%, 0.1% and 0.15%). AP and AN were obtained from the local market in Accra. They were cleaned, dried and blended. The spices were irradiated with dose of 10KGY to reduce microbial load. The experiment had three replicates each replicate was made up of 1b treatments. Six trained panelists evaluated the sausages using a 15 cm continuous scale on six sensory parameters (crumbliness, juiciness, palatability, saltiness, off flavour and overall liking), the formulated sausages with the selected local spices at varying concentrations did not differ ( $p \geq 0.05$ ) from the control product in all the sensory parameters. However, AP treated sausage at 0.15% was overall rated high. Culture of specimen-Aerobic bacteria, coliforms and *E. coli* were counted for the respective treatments stored for 0, 3, and 6 days at 4°C. Substituting nutmeg with AN up to 0.15% significantly ( $p \leq 0.05$ ) increased aerobic bacteria count. However, the substitution did not affect coliform and *E. coli* counts. Microbial count during storage at 0, 3 and 6 days at 4°C exhibited different growth patterns. For aerobic plate count, mean  $\log_{10}$  CFU/g count for aerobic bacteria and coliforms significantly increased between day 0 and day 3 and decreased from day 3 to 6. Coliform, mean log count similarly increased from day 0 to day 3. No significant ( $p$

□ 0.05) difference in log count was observed from day 3 to day 6. *E coli* log<sub>10</sub> CFU/g count decreased from day 0 to day 3, however, a significant increase in log count was observed from day 3 to day 6. The present study shows that *Xylopiya aethiopica* and *Monodora myristica* can be used to substitute for *Piper nigrum* and *Myritica fragrans* respectively, in the manufacture of fresh pork sausages without affecting the sensory attributes and overall liking of the product. However, the addition of such spices could alter microbial profiles significantly.



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## LIST OF ACRONYMS AND ABBREVIATIONS

APC	-	Aerobic Plate Count
BHA	-	Butylated hydroxyanisole
BHT	-	Butylated hydroxytoluene
CPF	-	Cryogenically Processed food
DLG	-	De La Grenada Industries Limited
EBO	-	Encyclopedia Britannica Online
EOs	-	Essential Oils
ERH	-	Equilibrium Relative Humidity
FDA	-	Food and Drug Administration
GI	-	Gastrointestinal
GIPC	-	Ghana Investment Promotion Centre
LIPREC	-	Livestock and Poultry Research Center
MIC	-	Minimum Inhibitory Concentration
MPL	-	Maximum Permissible Limit
NRCS	-	Natural Resources Conservation Services
NZFSA	-	New Zealand food safety Authority
TBA	-	Thiobarbituric acid
TBHQ	-	Ter-butylhydroquinone

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## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

Spices are dried seeds, flowers, fruits, barks, roots, leaves of plants or vegetative substances of different plants used in small quantities as food additives (Food and Agriculture Organization (FAO), 2010), and have been used in seasoning foods since time immemorial and at the same time serve as dietary supplements (Obadina and Ogundimu, 2011). Spices can improve the palatability; enhance flavour and visual appeal of dull diets. Piquant flavors are valued for other properties like stimulation of appetite, carminative and anti-oxidant effects and promotion of digestion (Takeda *et al.*, 2008).

Some chemical food additives however, have adverse side effect on human health (Tuula, 1994; Bridges and Bridges, 2007). Spices do not exhibit any toxicity at levels used and consumed (Oiye *et al.*, 2012), in view of these, spices have become the most ideal food additives. Pungent spices can cause sweating, which may result in a cooling sensation in tropical climates; on the other hand, they can add a sense of inner warmth when present in cooked foods used in cold climates (Takeda *et al.*, 2008). Research shows that deterioration of human health may correlate with food products and food additives (Tuula, 1994). Most importantly, spices are used in preservation of meat and meat products such as sausages.

Sausages play an important role in the nutrition of man. They are chopped or comminuted and seasoned meats that are formed into symmetrical shapes. They are more economical than whole meat cuts as they are produced from trimmings from primal cuts and by-products of meat (Jihad *et al.*, 2009). Sausages are the most appetizing and

widely utilized meat product among all processed meats, and are of many varieties depending upon mode of manufacturing (FAO, 2010). The main ingredients of sausages are lean meat, fat and additives such as salts, spices and seasoning. Preservatives used to prevent microbial spoilage and to enhance colour and flavour include inorganic chemicals, such as salt, nitrite or nitrate and sodium metabisulphite. Some ingredients which are locally unavailable, and need to be imported create production hitches (Oiye *et al.*, 2012).

Foods preserved with natural additives, which are generally recognized as safe have gained a wider interest in the meat industry for their potencies as antimicrobial and antioxidant e.g. oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage and vanillin (Annalisa *et al.*, 2012; Angioni *et al.*, 2004; Arques *et al.*, 2008; Daferera *et al.*, 2000; Davidson and Naidu, 2000; Gutierrez *et al.*, 2008).

The active components of spices and herbs are commonly found in the essential oil fractions. It is well established that most of them have a wide spectrum of antimicrobial activity, against food-borne pathogens and spoilage bacteria (Vijay *et al.*, 2012; Gutierrez *et al.*, 2008 ; Gutierrez *et al.*, 2009). Common spices used in sausage making include; paprika, mace, nutmeg, garlic, onion, sage, black pepper, white pepper, red pepper, ginger, rosemary, cinnamon and cloves.

Notably, most packaged spices imported by developing countries are exported in raw forms to developed worlds, where they are processed repacked and re-exported after significant value addition in the form of packaging and blending (SADC Trade, 2005) and decontamination by irradiation. According to FAO (1998), West and Central Africa

sub-regions have identified a large number of under-utilized species of plants that are important to livelihood of local populations. Some of these indigenous spices have been used in Ghanaian homes to prepare local dishes across the entire country, as flavour and taste enhancers (Dieu *et al.*, 2006). Even though these spices have been in use for centuries, there is inadequate information about their effects on nutritional value improvement, flavour enhancement and shelf life extension of meat products (Ekeanyawu *et al.*, 2010). Examples of these local spices include; African nutmeg and African pepper.

Sausage production in the developing world presents many problems, such as religious belief concerning pig and cattle consumption, while some people do not consume certain parts or organs of slaughtered animals. However, opportunities for profitable sausage production exist where meat resources are locally available, as livestock production and spices farming offers excellent raw materials for the product processing (FAO, 2010).

The ever-growing increased consumption of sausage in Ghana calls for the exploration of local spices in sausage manufacturing to cut down cost of production and make production more convenient. The growing consumer interest in 'ready- to- eat' meat products such as some varieties 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 with 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).

## 1.2 Problem Statement

In most countries, people prefer sausages made from beef and chicken. Pork suffers many negative attitudes because of religious practices and unwholesome perception attached to it (Antwi-Boateng *et al.*, 2013). Pork is not sold at the local market/meat shop that is under the control of Muslims because of their religious taboos. People consider pigs as dirty animals and as such their meat are unhealthy. Another factor crippling the pork industry is the high rate of importation of pork (Antwi-Boateng *et al.*, 2013). Meanwhile, demand for animal protein has increased in the world due to the increasing human population and it puts pressure on producers to improve efficiency of animal production. Although meat consumption figure in Ghana is low, sausage consumption on the other hand is on the increase (ADB, 2011). Besides, sausage manufacture does not only help minimize protein deficiency but also add value to pork and easier to consume by all including children and the elderly (Sebranek, 2003).

Most spices used in local sausage production are exotic. High cost of imported spices compared with local spices, lack of development of local plant resources of spice potential, cultivar selection, large-scale cultivation, processing and packaging for local and international markets and high loads of microbial contamination are major problems facing the spice industry in Ghana. Additionally, research to improve the acceptability and marketability of local spices is lacking. How much spices are imported annually? What is the cost to the economy of the nation? Are there potential substitutes? What is the current use of potential substitutes? Why have they not been used in sausage manufacturing? The development of new and improved quality spices will be beneficial to the food and meat industry. There is therefore the need to evaluate these selected local

spices on sensory and microbial characteristic of fresh sausage to cut down cost and promote local spices as well.

### **1.3 Justification**

The selected local spices are available in the local markets and affordable compared to imported ones. The results of this study will provide information on the potential use of local spices in sausage production.

### **1.4 Hypothesis**

African pepper and African nutmeg can be substituted for white pepper and nutmeg in sausage with no deleterious effect on sensory and microbial characteristics of fresh pork sausage.

### **1.5 Objective of the Study**

The objective of this study is to evaluate two selected local spices (*African pepper and African nutmeg*) on sensory and microbial characteristic of fresh pork sausage.

### **1.6 Specific Objectives**

The specific objectives of this study are to evaluate; (i) the effect of African pepper and African nutmeg on the sensory characteristics of fresh pork sausage (FPS); on significance. (ii) the effect of the spices on microbial load in sausages.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Sausage Development

Sausage manufacture evolved as a move or plan to economize and preserve meat that could not be consumed or utilized fresh at slaughter. The manufacturing of sausages by man began over two thousand years ago (Oscar, 1998). They are one of the oldest prepared foods, whether cooked or eaten immediately or dried to various degrees (FAO, 1985). In the olden days, people in the western world did not have refrigerator to preserve their meat hence sausage making was developed to overcome the problems of wastage and spoilage.

The modern word “sausage” is derived from the Latin word “*salsus*,” which means “salted” (Wijnker *et al.*, 2006). The preparation of sausages began with a simple process of salting and drying meats. Sausages have been produced from different meats such as beef, pork, chicken, fish and buffalo meat (Sachindra *et al.*, 2005). In addition, there are different types of sausages produced throughout the world based on the geographical location and the methods employed. Appendix1, described different sausages based on specific characteristics. For example; fresh sausage, dry and semi-dry sausage; cooked sausage; smoked sausages; and cooked meat specials (Rust, 1987 ; Romans *et al.*, 2001) and normally pork is chief meat for fresh sausages.

According to Holness *et al.* (2005), for a number of years, pork consumption in the developing world had shown a steady increase at the expense of beef. This in part could be attributed to the lower fat content and healthier image of pork. Orr and Shen (2006) revealed that, pigs are the main source of meat in the world (40%, 93 million metric

tons), China, European Union, United States, Brazil and Canada being the major producers and consumers.

In developing countries, pig production makes a significant contribution to household economies and food security. In addition, it also contributes to improving the nutritional status of the rural poor. Pigs yield on the average four to five times more meat than cattle per ton of live weight (Eusebio, 1980). When compared with cattle and other ruminants, pigs have some major potential advantages including their ability to produce meat without contributing to the deterioration of the natural grazing lands (Holness, 1991). Pigs are prolific because they are capable of producing large litters after a relatively short gestation period.

## **2.2 Importance of Pork as raw material in Sausage Manufacturing**

The benefits of pig production cannot be underestimated. Besides, domestic pigs can be found worldwide because of their extraordinary importance in the production of red meat, lard and cured products. Their production is one of the fastest ways of meat production as they have a short gestation period, large litter size and a source of employment as well as means of eradication of poverty especially the developing countries. Whittemore (1980) reported that, half of all the meat eaten in the world is from pigs. In 1993, pork and poultry meat, according to FAO (2001) represented 63% of all meat consumed globally. In 2011, pork was reported to be the most popular meat consumed in the world, totaling 37% of all meat eaten and produced, some 109 million tons worldwide (McDermott, 2012).

In Ghana, among the livestock, pigs hold immediate to short term solution to animal protein deficiency (Annor-Frempong and Segbor, 1994) since they are able to efficiently convert feed into edible meat (Barnes, 1994) including a variety of crop waste, kitchen waste and agro-industrial by-products into high quality meat.

Food from livestock and poultry such as milk, meat and eggs are rich in energy, protein, vitamins and micronutrients, which are particularly important in the diets of the most vulnerable groups, that is children, pregnant and breast feeding women. In developing countries, malnutrition is common. However, meat, milk and eggs provide an important opportunity to overcome this problem (Perry *et al.*, 2002).

Pork can be processed into various forms including sausage as a form of preservation and value addition. FAO (2010) revealed that sausages are the most appetizing and widely utilized meat product among all processed meats, and are of many varieties depending upon mode of manufacturing. Fresh pork sausage comprises the vast majority of fresh sausage produced throughout the world, and can be in bulk, link, or patty form. Fresh pork sausage is usually considered to be the third most popular processed meat product outranked only by wieners and bologna (Jay, 2011).

Fresh sausages are normally made from selected cuts of fresh meat; they may contain water not exceeding 3% of the total ingredients in the product. They are not cured and have to be stored under refrigeration or frozen state prior to their consumption (Romans *et al.*, 2001). Pork sausage can be manufactured from either chilled meat, trimmings from primal cuts or entire carcasses, or hot processed meat. To preserve the meat and improve the flavour of the product, salt is added and then flavoured with spices (FDA, 2010). They are then stuffed into casings (natural or artificial) to make the product

more convenient to eat. Studies have indicated a number of spices including garlic, rosemary, clove, black pepper, ginger, nutmeg, mace etc., which have been used in sausage making worldwide (FAO, 1985). The combination of spices depends on the taste, flavour, shelf life, colour among others, of the manufacturer. However, developing countries import most of these spices, which can be cultivated and processed for utilization.

### **2.3 Some Selected Spices and their Characteristics**

The name spice is derived from the word species, which was applied to groups of exotic foodstuffs in the middle ages, DeeSpice Limited (DSL) (2014). Spices are dried seeds, flowers, fruits, barks, roots and leaves added to foods for the purposes of flavour, colour, or as preservatives and limit the rate of rancidity (Thomas, 2007 and FAO, 2010). Spices are mostly tropical plants or parts valued for providing colour and aromatic flavouring along with stimulating odour for use in cooking and in condiments, as well as in candies, cosmetics, fragrances and medications (DSL, 2014). The most important natural spices used in meat products are pepper, paprika, nutmeg, mace, cloves, ginger, cinnamon, cardamom, chilli, coriander, cumin and pimento (FAO, 2010). Summary of the classification of natural spices based on the part used in food is shown in Appendix 2.

#### **2.3.1 Mace (*Myristica fragrans*)**

Mace, *Myristica fragrans*, is gotten from a tropical fruit known as nutmeg. Nutmeg is the seed of the tree, roughly egg-shaped and about 20 to 30 mm (0.8 to 1.2 in) long and 15 to 18 mm (0.6 to 0.7 in) wide, and weighing between 5 and 10 g (0.2 and 0.4 oz) dried, while mace is the dried "lacy" reddish covering or aril of the seed Encyclopedia

Britannica Online (EBO) (2014) ; Tropical Spice Garden (TSG) (2012). The tree is slow growing evergreen tree grows to more than 20 m and is cultivated in India, Ceylon, Malaysia, and Granada. Its fruit is called a drupe or a nutmeg apple, which is similar in appearance to a peach, or an apricot. The lacy aril is removed by hand from the outer shell of the nutmeg and then dried, becoming yellowish-brown mace, De La Grenada Industries Limited (DLG) (2009). Mace is sold in whole pieces called blades or in the more commonly found ground form. As it is dried, it develops its characteristic aroma but loses its bright red colour. The colour can often help you determine its origin. Orange-yellow blades most likely come from Grenada, while orange-red blades tend to be from Indonesia. Mace has a flavour described as a combination of cinnamon and pepper, a more pungent version of nutmeg. Unlike most spices, ground mace has a longer shelf-life when stored properly in a tightly-sealed jar or container in a cool dark place, Cryogenically Processed food (CPF) (2008).

### **2.3.2 White Pepper (*Piper nigrum*)**

White pepper, sometimes known as pepper-corn is from the seed of the plant *Piper nigrum*, which flesh or rind has been removed before milling the seed, P.C Kannan & Co (PCK) (2012). *Piper nigrum* belong to the *Piperaceae* family, Germplasm Resources Information Network (GRIN) (1995). They are mostly found in countries like India and Indonesia. Pepper berries look like grapes and they produce pepper corns in three kinds: white, black and green. They are sold either whole or ground. Black peppers are the most popular kind and they have the strongest flavour. They are processed before the pepper berry is even ripened. Green pepper corns come from berries that become immature and the seeds are processed by preserving them in brine. And finally, white pepper is processed from really ripened pepper berries. White pepper

has a slightly different flavour from black pepper due to the high content of piperine. It is also milder but more pungent in taste than the black kind (GRIN, 1995). White pepper's flavour is sometimes described as piney. White peppers are often treated using chemicals and enhancers to make them whiter in colour and is mostly sprinkled on food to preserve the appearance. Among all the spices, white pepper is the commonly natural spice used in sausage making (FAO, 2010). Spices are mainly used in the ground form, which improves visual appearance of the meat product. The processed particle size (from 0.1 to 1 mm) of the spice determines the release and distribution of flavour (Ockerman and Basu, 2004).

### **2.3.3 Onion (*Allium cepa*)**

The onion (*Allium cepa*), is also known as the bulb onion or common onion. It is used as a vegetable and is the most widely cultivated species of the genus *Allium* (Block, 2010). The onion plant is a perennial herb growing to about 1.2 m in height, with 4 to 6 hollow, cylindrical leaves. They are fleshy, hollow and cylindrical, with one flattened side. Onions contain 89% water, 1.5% protein, 2% fibre, 0.1% fat and vitamins B<sub>1</sub>, B<sub>2</sub>, C, and folic acid along with potassium and selenium, National Onion Association (NOA) (2011). They are pungent when chopped and contain alliinase enzyme, which acts on the chemical alliin converting it into alliin, that irritates the eyes (Kuettnner, 2002). Onions are good source of fibre, and with only 45 calories per serving, and add abundant flavour to a wide variety of food.

### **2.3.4 Marjoram (*Origanum majorana*)**

Marjoram is indigenous to Cyprus and Southern Turkey, it belongs to the *Lamiaceae* family (GRIN, 2012). Marjoram is cultivated for its aromatic leaves, which can be

steam-distilled to produce an essential oil. Marjoram can be used in seasoning soups, stews, dressings and sauce (Kains, 2007). *Origanum majorana* has exhibited some antimicrobial activities *in vitro* due to the presence of terpinen-4-ol (Busatta *et al.* 2008), marjoram reduced the count of *E. coli* in fresh sausages *in vitro*. It was concluded that marjoram could be used to prolong the shelf life of fresh sausages due to its bacteriostatic action at minimum inhibitory concentration (MIC) and its bacteriocidal action at higher concentrations (Mathenjwa, 2010).

### **2.3.5 Ginger (*Zingiber officinale*)**

Ginger is a herb but often known as a spice, from *Zingiberaceae* family. Ginger is indigenous to southern China, from whence it spread to other parts of Asia, and West Africa and to the Caribbean (Spices, 2002). It has a strong distinct flavour that can increase the production of saliva. It can be used fresh, dried and powdered, or as a juice or oil. The part that is used as spice on the plant itself is the rhizomes or ginger root. The ginger plant is approximately 30 – 60 cm tall and is extremely rare to find in the wild. Ginger produces clusters of white and pink flower buds that bloom into yellow flowers. Fresh ginger may be peeled before eating. Young ginger rhizomes are juicy and fleshy with a very mild taste. For longer-term storage, the ginger can be placed in a plastic bag and refrigerated or frozen. Ginger produces a hot, fragrant kitchen spice and acts as a useful food preservative. Ginger contains a very high level (3.85 mmol/100 g) of antioxidants (Halvorsen *et al.*, 2002). About 115 bioactive constituents are found in both fresh and dried ginger varieties. Gingerols are the major constituents of fresh ginger and are found slightly reduced in dry ginger; while the concentrations of shogaols, which are the major gingerol dehydration products, are more abundant (Jolad *et al.*, 2005). Several studies indicate that ginger suppresses lipid peroxidation and protects the levels

of reduced glutathione ( Reddy and Lokesh 1992; Ahmed *et al.*, 2000a; Ahmed *et al.*, 2000b; Ahmed *et al.*, 2008).

### **2.3.6 Clove (*Syzygium Gaertner*)**

Clove belongs to the family *Myrtaceae*. The plant is economically important due to its essential oils with strong fragrances, which can be used in several ways. A well-known example is pimentum (*Pimenta dioica*). Clove was originally named *Caryophyllus aromaticus* by Linnaeus but later transferred to the genus *Eugenia L.* by Thunberg however, clove is now known as *Syzygium Gaertner*. A number of characters in flower and fruit distinguish the exclusively old-World genus *Syzygium* from *Eugenia*, which mainly encompasses new world taxa.

Cloves thrive best with insular maritime climates in the tropics at low altitudes. In the original habitat in Moluccas, where the trees are semi-wild, annual rainfall is 218 – 355 cm and temperatures 24 – 33°C. Drier weather is desirable for harvesting and drying the crop. According to Murty and Subrahmanyam (1989), deep, sandy, red and acidic-loams are the best soils. Good deep drainage is essential. The tree is slender and can reach up to 15 m tall, conical when young, later becoming cylindrical, in cultivation usually smaller and branched from the base.

The bark is grayish smooth and the wood of stem is hard but brittle (Purseglove, 1968). Clove contains a volatile oil, 14 to 20%; gallotannic acid, 10 to 30%; oleanolic acid, vanillin, eugenin and other extraneous materials associated with dried buds (Robbers *et al.*, 1996). Clove is considered to be the most fragrant of all aromatic spices. Its flower

bud is mostly used as spice. Gustatory and sensory experts have described it to be “exquisitely scented, exhaling perfume rare and delicious”. The aroma of ground cloves has been characterized as being spicy, peppery, sweet, fruity, phenolic, woody and musty. According to Farrell, (1990), the aroma of cloves has been characterized as warm, spicy, fruity, astringent, and slightly bitter with a warm numbing effect. Cloves are usually used for seasoning sausages, sauces, apple pudding and tarts. According to Cai and Wu (1996) there is clear evidence that clove exhibits antibacterial activity against important oral pathogens and may therefore be an important prophylactic. Hot water extract of *Syzygium aromaticum* showed anti-cytomegalovirus (CMV) activity in vitro. When orally administered to mice, the activity of cytomegalovirus was also suppressed in the lungs (Yukawa *et al.*, 1996).

### **2.3.7 African Pepper (*Xylopi aethiopica*)**

*Xylopi aethiopica*, commonly known as african pepper, guinea pepper, Spice tree, ethiopian pepper belong to the family Annonaceae. It is locally known as, etso, soo, hwentia, chimba, kimba, (Ewe, Ga, Akan, Hausa and Dagbalin respectively) and consist of dried mature fruit and has characteristic odour and taste (Orwa *et al.*, 2009). It is a slim, tall, evergreen, aromatic tree up to 15–30 m high and about 60–70 cm in diameter with straight stem, many-branched crown and sometimes buttressed. It often has short prop roots, smooth grey-brown bark and scented when fresh. Its leaves are simple, alternate, oblong, elliptic to ovate, 8-16.5 by 2.8-6.5 cm, leathery, bluish-green and without hairs above, but with fine brownish hairs below, margin entire, and glabrous. Its petiole is about 0.3-0.6 cm, thickset and dark-coloured. Flowers of the tree are bisexual, solitary or in 3-5 flowered fascicles or in strange, sinuous, branched spikes, or cymes, up to 5.5 by 0.4 cm and creamy-green. The fruits of *Xylopi aethiopica* are

small with carpels of about 7-24, forming dense cluster. The fruits have dark brown colour when matured, cylindrical and are about 1.5-6 cm long and 4-7 mm thick (Tairu, *et al.*, 1999). The contours of the seeds are visible from outside.

Extracts of the fruits, or decoction of the bark, have been reported to have wide ethno-medicinal uses. In Ivory Coast the fruit extract is used as tonic to encourage female fertility, for ease of childbirth and as a remedy for woman after childbirth (Burkill, 1985). They are useful in the treatment of bronchitis and dysenteric conditions, and also as a medicine for biliousness and febrile pains.

In Congo, the bark is steeped in palm-wine which is given for attacks of asthma, stomach-aches and rheumatism at the rate of one or two glasses per day (Acquaye *et al.*, 2001). The wood is resistant to termite attack and is used in hut-construction for posts, scantlings, roof-ridges and joists most especially in Togo and Gabon. It burns with a hot flame and has found use as a steamboat fuel. The root-wood can be used as a cork. The root is strongly aromatic and a concentrated root-decoction is used as a mouthwash for toothache in Africa (Burkill, 1985). The seeds, as separate from the fruit, are substitute for pepper, and have cosmetic, revulsive and stimulant uses.

The powdered root is used as a dressing for sores and to rub on gums for pyorrhoea and in local treatment of cancer in Nigeria, and when mixed with salt is a cure for constipation. The dry fruits are an important spice used to prepare local dishes and soups in West Africa (Acquaye *et al.*, 2001). The chemical constituents of *Xylopiya aethiopiya* include essential oils, resins, annonacin, reberoside, avicien, rebersole, alkaloids, tannins, oxalate, and flavonoids (Ekeanyanwu and Etienajirhevwe, 2012). *Xylopiya aethiopiya* extracts demonstrated antimicrobial activity against gram positive and negative bacteria,

however, it has not been shown to be effective against *E. coli* (Iwu 1993). *Xylopic* acid has also demonstrated activity against the fungus *Candida albicans* (Acquaye *et al.*, 2001).

### **2.3.8 The African Nutmeg (*Monodora myristica*)**

The African nutmeg (*Monodora myristica*), also known as calabash nutmeg belonging to the family Annonaceae or custard apple family of flowering plants (Okafor, 2003). It is locally known as awerewa, ayikue, ariwo (Akan, Ewe and Ga) etc. The tree grows naturally in evergreen forests from Liberia through Ghana, Nigeria and Cameroon, Angola and also Uganda and west Kenya. The tree can reach a height of 35 m and 2 m in diameter at breast height. Several studies reveal that almost every part of the tree is of economic importance (Okigbo and Igwe, 2007; Okafor, 1987). However, the most economically important part is the seed, which is embedded in a white sweet-smelling pulp of the sub-spherical fruit. The odour and taste of the *Monodora myristica* seed is similar to nutmeg. The kernel is a popular condiment used as a souring agent.

When ground into powder, the kernel is used to prepare all kind of soups and as stimulant to relieve constipation and control passive uterine haemorrhage in women immediately after childbirth (Burubai, 2009). In addition, the essential oils extracted from the roots, seeds and stem barks of *Monodora myristica* is used to cure scabies, toothache, helminthiasis, malaria, dysenteric syndromes, constipation and as a stimulant (Talalaji, 1999; Irvine, 2000; Okpekon *et al.*, 2004). The seeds contain 5-9% of a colourless essential oil. The major compounds found in the essential oil from the seeds are  $\alpha$ -phellandrene,  $\alpha$ -pinene, myrcene, limonene flavonoids, phenols, tannins, saponins and pinene. The plant's proximate composition ranged from 3.06 to 52.42% with nitrogen free extract having the highest value and ash content lowest (Ochuko, 2012).

Appendix 3 gives the detail of the proximate composition of *Monodora myristica*. According to Ogu *et al.* (2011) *S. aureus*, *E. coli*, *P. aeruginosa*, *B. cerus*, *P. mirabilis* and *C. albicans* susceptibility to african nutmeg extracts' increased at 200 mg/ml. The zone of inhibitions of *Monodora myristica* extracts against *Escherichia coli* was (11millimetre), *Bacillus substilis* (8millimetre) and *Staphylococcus aureus* (11millimeter) (Adewole *et al.*, 2013). *Monodora myristica* exhibited high antioxidant activities *in vitro*, signifying the protective potential of the spice against free radicals (Ochuko, 2012), and this may be attributed to phenolic and flavonoids content of the spice. According to Ochuko *et al.* (2012) phytochemicals screening of *M.myristica* revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, terpenoids and saponins Appendix 3, which may contribute to its antioxidant activities.

#### **2.4. Uses of Spices**

Spices are mainly used to improve the palatability/ taste and the visual appearance of diets. Spices piquant flavors stimulate salivation and promote digestion. They also improve the texture of some finished products. Spices, along with salt, in mixtures seem to preserve meats with a great taste. Pungent spices were useful for relieving the salty taste of such foods, Shiny Spices (SS) (2013). The aromatic spices such as cinnamon, cloves, mint etc. are used to disguise the foul breath from onion and garlic. In seasoning of meat and fish, spices enhanced the meat or fish flavour (SS, 2013). A spice's flavour strength and quality are subject to variation depending on season, where and how it is grown, handling, transportation and processing (Ockerman and Basu, 2004).

The production process of Natural spices can also be a source of microorganisms' contamination in food (Ockerman and Basu, 2004; FAO, 2010). The most common

methods employed in decontaminating microbes on spices include radiation techniques such as gamma, electronic, UV or heating, microwave processing, exposure to steam and steam distillation and treatment with ethylene oxide gas or high-pressure processing. Most spices have antimicrobial and/or antioxidant properties, which served as food preservatives. The antimicrobial and antioxidant activities of plant essential oils is due to their chemical structure, in particular to the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components, flavanoids, terpenoids and lipophilicity of some essential oil components (Craig, 1999).

‘Essential oils’ (EOs) are volatile, odoriferous components that are present in many plants and are normally obtained by steam distillation (Ockerman and Basu, 2004). The most components of EOs are hydrocarbons (terpenes, sesquiterpenes), oxygenated compounds (alcohol, esters, aldehydes, ketones) and non-volatile residues (waxes, paraffin). Essential oils only contain part of the spice profile and are often deficient in bitterness, heat, sweetness, and other flavour components whiles oleoresins’ are prepared from spices or herbs by extraction with organic solvents and contain both the volatile portion (essential oils) and the non-volatile extract, which includes resins. Oleoresins contain more complete flavour profile than essential oils. Both oleoresins and essential oils are microbiologically sterile and can be standardized for strength and flavour profile (Ockerman and Basu, 2004).

#### **2.4.1 Flavour Enhancer**

Flavourants are agents that are intentionally added to food with the sole intention of imparting flavour. There are several different categories of flavouring agents such as smoke flavours and process flavourings (European Commission, 1988). Flavouring

substances are organic volatile chemicals, with a molecular weight not exceeding 300 g/mol and composed of carbon, hydrogen, often oxygen and sometimes nitrogen or sulphur but never any other element unless the substances are salts with common physiological cations or anions.

#### **2.4.2 Antimicrobial Properties of Spices**

Comminuted processed meat such as sausages contain meat and fat as major ingredients. This provides a medium for spoilage microbial growth and fat deterioration, which affect both shelf life and organoleptic quality of the product. Shelf life is a guide for the consumer of the period of time that food can be kept either under refrigeration or under room temperature before it starts to deteriorate, provided all storage conditions have been followed (Microchem, 2007). The shelf life of a product begins from the time the food is produced. According to New Zealand Food Safety Authority (NZFSA) (2005), shelf life duration is dependent on many factors such as the types of ingredients, processing, type of packaging and how the food is stored. The technology employed in sausage production is said to affect the type of microbial contamination, which might gain access into the sausage from either the meat or the natural casing or the spices and other non-meat ingredients. In addition, production handling/poor personal hygiene, poor environment and equipment also serve as sources of microbial contamination. It is therefore important to incorporate preservative agents into meat products to control microbial spoilage and oxidation of fat.

Additionally, the use of spices as preservation technique reduces the use of chemical food additives such as nitrite and salt in meat products. Meat products are normally preserved by heat treatment to 75 °C in the product, thereby eliminating all the live microbes in the product. Products that are not heat-treated to 75 °C, the growth

inhibition are achieved by low pH value obtained by fermentation with lactic acid bacteria in combination with a rather high amount of salts (for instance salami, pepperoni etc.). However, excessive intake of salt correlated with increased risk of coronary heart diseases and an increased high blood pressure Consensus Action on Salt and Health (CASH) (2008). This has led consumers to perceive natural antimicrobial preservatives as a healthier alternative to synthetic antimicrobial preservatives.

Research from the 1940's to late 1970's created the concerns that consuming food treated with nitrate and nitrite could have adverse effect on human health and at times death .In response, meat processors began utilizing natural nitrate sources such as celery powder and many more (Gary, 2011). Spices are often strong, zesty, pungent, fiery and fragrant, giving a dish an exotic and exciting taste and play a major role in culinology, as they are versatile in feature (Rathore and Shekhawat, 2008). More importantly, most spices contain dozens of secondary compounds and these chemical compounds help to protect them against herbivorous, insects and vertebrates, fungi, pathogens, and parasites (Walker, 1994). Some animals that store food often add plants with antibacterial and antifungal properties to their caches e.g., brown bears sometimes cover carcasses with Spaghnum moss (Elgmork, 1982). Additionally, onion has been used to prevent the growth of oral pathogenic bacteria, including *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* organisms associated with dental caries and periodontitis (Sarika and Shikha, 2014). The antimicrobial ability of onion is associated with a number of sulfur containing compounds such as alliin, allylalliin, diallyl disulfide and the methyl and propyl compounds of cysteine sulfoxide Natural Resources Conservation Services (NRCS) (2007). Onion oil also inhibited growth of other gram-positive bacteria and gram-negative bacteria such as *Klebsiella pneumoniae*. Antifungal

actions of onion include inhibition of yeasts and a number of molds (Sarika and Shikha, 2014). Garlic extracts have demonstrated antifungal activity when tested in vitro suggesting a potential use in the treatment of oral and vaginal candidiasis. Several studies revealed garlic possessed wide spectrum of antibacterial, antiviral, antifungal and antiprotozoal activities and also for its medical benefits on the immune and cardiovascular systems (Sallam *et al.*, 2004). Garlic oil and oregano were effective against species such as *S. aureus*, *S. enteritidis*, *L. monocytogenes*, *E. coli* and *L. plantarum* in whey protein-based film (Coma, 2008). According to Martinez *et al.* (2007), fresh garlic and the powder has been shown to maintain aerobic plate count in chicken sausage below the maximum permissible limit (MPL) stored at 3 °C (MPL is 7 log<sub>10</sub> cfu/g). At 30 g/kg and 9 g/kg, fresh garlic and powder garlic produced significant antimicrobial and antioxidant effects and extended the shelf life of chicken sausage up to 21 days (Sallam *et al.*, 2004).

According to Matan *et al.* (2006), the combination of cinnamon and clove oils suppresses the growth of major spoilage microorganisms of intermediate-moisture foods. The eugenol in clove oil has shown a strong inhibition effect on the growth of *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus* in various agar mediums (Mytle *et al.*, 2006). At about 2000 µg/ml, ginger had shown minimum inhibitory concentration (MIC) effect against *Clostridium botulinum*.

The EOs in ginger (gingerone and gingerol) showed inhibitory effect against both cholera and typhoid bacteria (Hirasa and Takemasa, 1998). Gingerols has shown other antimicrobial properties against *B. subtilis* and *E. coli* (Yamada *et al.*, 1992) and *Mycobacterium* (Galal, 1996). Enterocin production by *Enterococcus faecium* CTC

492 was strongly reduced when black pepper was added to the fermentation medium (Aymerich *et al.*, 2000). Spices such as oregano, rosemary, thyme, sage, basil, turmeric, ginger, garlic, nutmeg, clove, mace, savory, and fennel, have been successfully used alone or in combination with other methods of food preservation. They either exert direct or indirect effects to extend food product shelf life or as antimicrobial agent against a variety of Gram positive and Gram-negative bacteria. Meanwhile, their efficacy depends on the pH, the storage temperature, the amount of oxygen, the EO concentration and active components (Gutierrez *et al.*, 2008a; Sandasi, *et al.*, 2008; Viuda-Martos, *et al.*, 2008).

Terpenes, carvacrol, p-cymene, and thymol, are the active compounds in oregano, savory and thyme that have demonstrated antifungal and antimicrobial activity that has attracted attention recently because of their potential food safety applications, especially for oregano (Bendahou *et al.*, 2008). The five most common antimicrobial compounds are  $\alpha$ -Pinene, cineole, limonene, linalool and geranyl acetate that have been effective against some food-borne pathogens and antibiotic-resistant *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Campylobacter jejuni* (Chen *et al.*, 2008; Sandasi *et al.*, 2008). According to Bañón *et al.* (2007), at a maximal tolerated concentration (MTC), grape seed extract exhibits a high antioxidant activity in fish oil and frozen fish, cooked pork patties and cooked turkey.

#### **2.4.3. Antioxidant Activity of Spices**

Antioxidants are substances that at low concentrations retard/inhibit the oxidation of easily oxidizable biomolecules, such as lipids and proteins in meat products, thus improving shelf life of products by protecting them against deterioration caused by

oxidation (Liz and Getty, 2012). According to Pavana *et al.* (2009), antioxidants protect the body against adverse effects of generated reactive oxygen species, which possess the ability to cause oxidative damage to the human body. Oxidation of lipid is influenced by the composition of phospholipids, the amount of polyunsaturated fatty acids, the presence of metal ions, oxygen, haem pigments, mechanical processes, and salt addition to product during processing. Lipid oxidation occurs when polyunsaturated fatty acids react with molecular oxygen via free radical chain mechanism forming peroxides (Devatkal *et al.*, 2010).

Oxidation of lipid is one of the major causes of food quality deterioration, and has economic importance (Raghavan and Richards, 2007) as it can negatively affect sensory attributes such as colour, texture, odour and flavour as well as the nutritional quality of the product (Nunez de Gonzalez *et al.*, 2008). Antioxidants play major role in food preservation due to their ability to prevent oxidative deterioration of lipids (Ademiluyi and Oboh, 2008) and this has led to the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) (Jayathilakan *et al.*, 2007), as antioxidants in meat products. These synthetic antioxidants have fallen under serious scrutiny due to their potential toxicological effects (Naveena *et al.*, 2008; Nunez de Gonzalez *et al.*, 2008). As a result, the meat industry is in a need of economical and effective natural antioxidants that can replace synthetic antioxidants without any negative effect on finished products quality and consumer perceptions on health. Several studies have focused on identification of novel antimicrobial and antioxidants from natural sources. This is mainly due to their high content of phenolic compounds, which make plant materials a good source of natural antioxidants providing an alternative to currently used

conventional antioxidants (Sanchez-Moreno, 2002; Nunez de Gonzalez *et al.*, 2008). Natural antioxidants such as rosemary and spice extracts have been reported to be more active than synthetic antioxidants. According to Schuler (1990), antioxidants properties of certain ground spices stabilized fat in food products. The carnosic acid in rosemary has been described as the most active antioxidant constituents (Brieskorn and Domling, 1969). Antioxidant extract from rosemary are commercially available in fine powder forms. Depending on the active content of antioxidant extract from plants, the recommended levels range between 200 and 1000mg/Kg of finished product (Schuler 1990).

The European Union in 2010 authorized the use of rosemary extracts as new food additives to be used in foodstuffs under Directive 95/2/EC and assigned E 392 as its E number (European Union directives 2010/67/EU and 2010/69/EU). This approval of carnosic acid and carnosol rated rosemary extract as a safe natural alternative to synthetic antioxidants. Garlic extracts have also shown antioxidant activity in different in vitro models. The antioxidant activity of *Allium* spp. bulbs has been attributed to a variety of sulphur-containing compounds and their precursors (Aarni *et al.*, 2003). Curry leaf extracts which contain monoterpene derived hydrocarbons and alcohols have been recognized for their antioxidant efficacy in foods (Ningappa *et al.*, 2008 ). Bali *et al.* (2011) indicated that coriander treated samples exhibited better antioxidant property where as garlic treated samples showed a better antimicrobial property.

#### **2.4.4. Medicinal Properties of Spices**

Spices were believed to improve health, since it was understood that they could affect the four humours (blood, phlegm, yellow bile and black bile) and influence the

corresponding moods (sanguine, phlegmatic, choleric and melancholic). Ginger is used to heat the stomach and improve digestion; clove is believed to comfort the sinews; mace helps to prevent colic and bloody fluxes or diarrhea; nutmeg help relieve any bad cold. A 15% tincture of cloves is effective in treating topical fungal, ringworm infection (Sarika and Shikha, 2014). Cinnamon oil has been used to combat microorganisms, diarrhea and other GI disorders, and dysmenorrhea.

According to Saudi (2008) ginger has a significant lipid lowering effect on patients with hyperlipidemia. The aromatic EOs of five plants (*Eucalyptus citriodora*, *Eucalyptus globulus*, *Mentha piperita*, *Origanum syriacum*, and *Rosmarinus officinalis*) combined on patients with upper respiratory tract infections were relived on day six (Eran *et al.*, 2011). Ten milligrams of dried oregano leaves administered as an oral solution to calves with diarrhoea was effective in the treatment of colibacillosis as neomycin (Bampidis *et al.*, 2005).

Turmeric is used to cure cancer as it has anti-cancer agents known as curcumin. It kills bacterial and reduces fungus growth at concentrations  $>100 \mu\text{M}$  (Lüer *et al.*, 2010). Curcumin which gives turmeric its yellow color, help people fight off or prevent diseases such as diabetes and cancer (Sundaram, 2005). Mace has been used extensively in treatment of wide range of ailments such as digestive disorder, rheumatism, cholera and flatulence (Shibamoto and Bjedanes, 1993). Tea and grape seed extracts have been suggested to add nutraceutical and health benefits (Jayaprakasha *et al.*, 2003).

## 2.5 Microbial Composition of Fresh Sausage

Meat generally is a nutrient-dense medium ideal for growth of a wide range of microorganisms (Garbutt, 1997; Russo *et al.*, 2006; Zhang *et al.*, 2009) especially comminuted meat, which possesses a large surface area for easy contamination during the mixing or mincing operation, American Meat Institute Foundation (AMIF) (1997). The major types of these microorganisms found in meat and meat products are bacteria, yeasts and moulds. Garbutt (1997) and Huffman (2002), studies showed that the composition of meat microbiota associated with spoilage of fresh meat includes *Enterobacteriaceae*, *Lactobacilli*, *Pseudomonas*, *Brochothrix thermosphacta* and *Shewanella putrefaciens*.

Spoilage microorganisms can readily grow in both fresh and precooked meat products, rendering the product inedible and unattractive by destroying the colour, flavour and structure. Surface spoilage of sausages may be influenced by high moisture content, and is normally shown by the appearance of slime, due to excessive bacterial proliferation, or by an abundant greyish-white growth of mould (FAO, 2010). Food and meat products can be categorised as perishable, semi-perishable and stable or non-perishable products (Madigan *et al.*, 2003). Fresh sausages have been categorised as perishable food products due to their high water activity and pH (Romans *et al.*, 2001; Cocolin *et al.*, 2004). Spoilage occurring in the interior portion of the sausage is usually due to adverse effect of unhygienic production conditions as well as poor hygienic quality of processed raw materials. According to Borch and Nerbrink (1989); Dykes *et al.* (1994); Dykes and Von Holy (1993); Korkeala and Mäkelä (1989), great diversity of lactic acid bacteria can be found in different types of spoiled cooked sausages.

### 2.5.1 Spoilage Bacteria

The microbial composition of meat and meat products may depend mainly on the kind of environment, the type of meat and raw materials, equipment, packaging materials and storage temperature (Sachindra *et al.*, 2005). Carcass can be contaminated with bacteria from the water, air and the soil as well as from the faeces and the intestine of animals, the workers, and the equipment used during the manufacturing process, after slaughtering (Sachindra *et al.*, 2005). Microbial spoilage is the most common cause of spoilage, and it is visible through the growth of colonies and slime, textural changes (degradation of polymers) or as development of off-flavours. According to Cocolin *et al.* (2004), microbial profiles of fresh sausage are characterized by the presence of aerobic, facultative anaerobic and mesophilic bacteria, which cause spoilage and food poisoning. The count range of aerobic colonies range from  $1.5 \times 10^3 - 2.1 \times 10^8$  cfu/g for fresh sausage and for frozen sausage from  $1.4 \times 10^3 - 3.1 \times 10^7$  cfu/g (Farber *et al.*, 1988).

One of the hygiene indicators of meat and meat products is the presence of *Enterobacteriaceae*. According to Crowley *et al.* (2005), the presence of *Enterobacteriaceae* on processed meats is better indicator of poorly treated or post-process contamination from the environment than coliforms since it is a direct indicator of the extent of faecal contamination. High number of *E. coli* present in meat and meat products is an indication of the presence of organisms of faecal origin. Nel *et al.* (2004) suggested that the maximum limit of *E. coli* in meat and meat products such as sausage should not be more than 10 cfu/g. As carcasses are moved into the chill room at near 0°C, a selective environment is developed that favours growth of psychrophilic microorganisms such as *Pseudomonas*, *Moraxella* and *Acinetobacter*; the three; most dominant genera of psychrotrophic microbes of soil origin. They are gram negative,

strictly aerobic rods that are able to grow at 0°C and even slightly lower. *Pseudomonas* spp. causes spoilage of meat and meat products stored at low temperatures (Ercolini *et al.* 2007; Olofsson *et al.*, 2007). *Pseudomonas fragi* is the most dominating specie of *Pseudomonas* that causes spoilage of meat stored in a refrigerator (8°C or lower), followed by *Pseudomonas lundensis* and *Pseudomonas fluorescens* (Olofsson *et al.*, 2007). *Pseudomonas* strains grow rapidly and will predominate if chilled moist meat is held longer. The growth of *Pseudomonas* spp, is affected by oxygen tension, salt, water activity ( $a_w$ ) and pH. *Pseudomonas* produces proteases and lipases which can catalyze reactions causing degradation of protein and fat during their growth period. They produce malodorous substances (esters, sulphides, acids) from amino acids metabolism (Pittard *et al.*, 1982).

### **2.5.2 Food –Borne Pathogens.**

The leading causes of illness and death in less developed countries are food borne pathogens, killing approximately 1.8 million people annually. (Sheng-Quan *et al.*, 2009). Most comminuted meat products are full of many types of pathogenic microbes such as *Salmonella* spp., *S. aureus*, *Listeria monocytogenes*, *E. coli* and *Campylobacter jejuni* (Eisel *et al.*, 1997). *Escherichia coli* serotype O157:H7 is a gram-negative, rod-shaped bacterium. The "O" refers to the cell wall antigen serological classification, whereas the "H" refers to the flagellar antigen (James and James, 1998). It is frequently detected in the intestinal tracts and hide of cattle and pigs (Mathenjwa, 2010). It can be transmitted through consumption of contaminated food and water with faecal matter. (Li and Logue, 2005; Arthur *et al.*, 2008; Ateba and Bezuidenhout, 2008; Wong *et al.*, 2009). *E. coli* O157 outbreak was attributed to undercooked ground beef patties sold from a fast-food restaurant, which have seen *E. coli* O157 and became broadly recognized as an important

human pathogen (Bell *et al.*, 1994). *Enterobacteriaceae* are aerobes or facultative anaerobes that ferment a wide range of carbohydrates, possess a complex antigenic structure and produce a variety of toxins and other virulence factors (Sharma and Adlakha, 1996). They are a large heterogeneous group of Gram-negative rods flora of the large intestinal tract of both humans and animals. The family includes genera *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *etc.*

A study conducted by Sharma and Adlakha (1996) shows that fecal coliforms are bacteria that ferment lactose to produce acid and gas at 44.5 °C up to 48 hours and hence produce bad odours. *Staphylococcus aureus* belongs to the phylum *Firmicute*, and is commonly found in the respiratory tract and on the skin of both human and animals. It is a facultative anaerobic Gram-positive coccal bacterium, also known as "golden staph" and Oro staphira (Kluytmans *et al.*, 1997). These bacteria are present in 20 to 30 percent of healthy people and are even more common among those with skin, eye, nose, or throat infections (Ford and Paula, 2005). This species is relatively tolerant to salt, which is found in some cured meat products as an essential selective medium for its growth when the moist cut surface is contaminated (Price and Schweigert, 1987). *Staphylococcus aureus* can grow extensively and produce the heat-stable enterotoxin without any resultant off flavours or odours. It is a common cause of skin infections (example boils), respiratory disease (example sinusitis), and food poisoning. The species can grow at a lower pH aerobically than anaerobically (Price and Schweigert, 1987). A study conducted by Barber and Deibel (1972) observed this growth characteristic in some fermented sausages.

## 2.6 Factors Influencing Shelf Life of Sausages

Spoilage of food is generally influenced by environmental factors such as the food matrix, microbial characteristics, and water activity ( $a_w$ ), temperature, pH, processing time, processing technology, etc. Sausages have high water activity and high concentrations of readily available nutrients that made them susceptible to microbial growth, leading to development of off-odours, off-tastes and changes in texture and slime formation (Xiang and Holley, 2012). Intrinsic factors are factors that are characteristic of the food itself such as pH and free moisture and extrinsic factors are those that relate to the environment surrounding the food such as temperature and storage atmosphere (FDA, 2013).

Generally meat and meat products spoilage is characterized by, (i) bacterial growth and metabolism causing formation of objectionable compounds, such as off-odours, gas, and slime, and (ii) lipids and pigments oxidation causing undesirable flavours and discolouration (Xiang and Holley, 2012). The numbers of microorganisms on fresh meat surfaces during chill storage change their typical growth pattern (Garcia-Lopez *et al.*, 1998) in response to temperature, pH, and available oxygen. However, only around 10% of these initial bacteria present are capable of growth during refrigeration storage of meat. Usually the nature and extent of surface contamination of meat determines its potential shelf life.

### 2.6.1 Effect of Moisture Content of Spices on Shelf life Meat Product

Water activity is the ratio of water vapour pressure of the food substrate to the vapour pressure of pure water at the same temperature (Jay, 2000); ( $a_w = p/p_o$ , where  $p$  = vapour pressure of the solution and  $p_o$  = vapour pressure of the solvent (usually water). The

$a_w$  of pure water is 1.00 and the  $a_w$  of a completely dehydrated food is 0.00 (FDA, 2013). The  $a_w$  of a food on scale from 0.00 - 1.00 is related to the equilibrium relative humidity above the food on a scale of 0 - 100%. Thus, percentage Equilibrium Relative Humidity (ERH) =  $a_w \times 100$ . The  $a_w$  of a food describes the degree to which water is "bound" in the food, its availability to participate in biochemical reactions, and its availability to facilitate growth of microorganisms (FDA, 2013).

The water activity of a food is a measure of its ability to support the growth of food spoilage microbes. Foods that are fresh have  $a_w$  values that are close to the optimum growth level of most microbes (0.97 - 0.99) such as fresh meat, fresh sausages, vegetables and fruits. Water activity has a decisive influence on the growth of microbes in food (Claudio, 2008 ; FAO, 2010). The amount of water in sausages has a direct effect on microbial, chemical and enzymatic reactions (FAO, 2010). The  $a_w$ -value of lean meat is about 0.99. In traditional sausage making,  $a_w$  is reduced by drying or by addition of curing salts, non-ionic solutes, such as sugar, spices and different food additives (Claudio, 2008; FAO, 2010). The  $a_w$ -value can be influenced by appropriate formulation of the sausages as well as by manufacturing methods. For instance, water, fat and salt contents directly influence  $a_w$ -value of sausage. Thus, one percent level of fat added to the formulation will reduce the  $a_w$ -value by 0.00045 and one percent of sodium chloride will also reduce the  $a_w$ -value by about 0.0060 while one percent of sugar and that of soy or milk protein will reduce the  $a_w$ -value by about 0.0020–0.0025 and 0.0012–0.0013 respectively (FAO, 2010). The optimum and minimum levels of  $a_w$  for microbial growth depends on several growth factors in their environments, such as cell wall characteristics of microbes which determine their sensitive to low  $a_w$ . For instance, Gram negative bacteria generally are more sensitive to low  $a_w$  than Gram positive bacteria (FDA, 2013).

Many bacterial pathogens growth can be controlled at water activities below 0.86 and only *S. aureus* can grow and produce toxin below  $a_w$  0.90. Multiplication of bacteria and yeasts is basically stopped at  $a_w$ -values lower than 0.88 to 0.90 (FAO, 2010; FDA, 2013).

Water activity influences deteriorative chemical reaction rate as water acts as a solvent; it can be a reactant on itself or can change the mobility of reactant through viscosity. Any one or a combination of any of these factors can lead to faster deterioration and shortened product shelf life AquaLab University (ALU) (ALU, 2014).

### **2.6.2 Influence of Nutrient Source on Microbial Growth**

Microorganisms need basic nutrients for their growth and maintenance of metabolic functions. The type and amount of nutrients needed depends greatly on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins, and minerals (Mossel *et al.*, 1995; Ray, 1996; Jay, 2000). Meats have abundant protein, lipids, minerals, vitamins and low levels of carbohydrates (Cross and Overby, 1998). Some bacteria metabolize more carbohydrates that are complex such as glycogen found in muscle foods and others can also use fats as an energy source (FDA, 2013).

Gram positive bacteria are generally more fastidious in their nutritional requirements; therefore, they are not able to synthesize certain nutrients required for their growth (Jay, 2000). *S. aureus* is one of the gram positive food borne pathogen that requires amino acids, thiamine and nicotinic acid for growth. However, the gram negative bacteria generally, are able to derive their basic nutritional requirements from the existing carbohydrates, proteins, lipids, minerals, and vitamins that are found in a wide range of food (Jay, 2000). *Salmonella enteritidis* is one example of food borne pathogens with

specific nutrient requirements. The availability of iron may limit the growth of *Salmonella enteritidis*. Thus, the availability of essential nutrients limits the rate of microbial growth in foods.

### 2.6.3 pH and Acidity

pH is a function of the hydrogen ion concentration in the food:  $\text{pH} = -\log_{10} [\text{H}^+]$ . Many bacterial pathogens grow best at pH values near neutral (i.e., 6.6-7.5) (Jay, 2000). It is a common method used to extend the shelf life of products. At a low pH values growth of foodborne pathogens bacterial can be inhibited and in some cases kill such pathogens. Acidity is a key factor affecting the growth and survival of microorganisms in food.

A food's ability to support the growth and survival of spoilage pathogens is influenced by its pH. The pH of food products vary with time due to microbial activity and product formulations. The pH range for microbial growth and survival is determined by a minimum and maximum value with an ideal pH for optimum growth and survival. Most microbes grow best at or near a neutral pH value. A pH value of 4.6 or less is important in food processing as it prevents *Clostridium botulinum* from producing toxins and is the basis for commercial sterilization in low and intermediate acid foods Food Safety Authority of Ireland (FSAI) (2011).

### 2.6.4 Temperature

Temperature is an extrinsic key factor affecting growth of food borne pathogens. Meat and meat products require low storage temperatures to retard the growth of microorganisms between -1 to +4°C by chilling or between -18 to -30°C freezing (FAO, 2010). Meats meant for sausage processing can be processed at these temperatures to inhibit bacterial growth. All microbes have a defined temperature growth range, thus a

minimum, maximum, and optimum. Temperature has an adverse effect on both the generation time of an organism and its lag period; the growth rate of an organism is defined as an Arrhenius relationship (Mossel *et al.*, 1995). There are four major groups of microorganisms that have been described based on their temperature ranges for growth: thermophiles, mesophiles, psychrophiles and psychrotrophs, International Commission on Microbiological Specification for Foods (ICMSF) (1980). The lag phase and growth rate of a microorganism are influenced not only by temperature but by other intrinsic and extrinsic factors such as pH,  $a_w$  and time.

### **2.6.5 Impact of Time**

Time is another critical extrinsic factor concerning the growth rate of microbial pathogens in food (FDA, 2013). Food producers address the concept of time as it relates to microbial growth when a product's shelf life is determined. Time can be used under certain circumstances at given ambient temperatures to control product safety. This can be achieved when the duration is equal to or less than the exponential phase of the pathogen(s) of concern in the product. The lag phase for growth of *L. monocytogenes* at 10 °C (50 °F) is 1.5 d, while at 1 °C lag phase is ~3.3 d. Likewise, at 10 °C the generation time for the same organism is 5-8 h, while at 1 °C (34 °F), the generation time is between 62 and 131 h (Mossel and Thomas, 1988). Time and temperature are integral, when considering growth rate of microbial pathogens in food.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1. The Study Area

The study was conducted at the Meat Science Laboratory and Microbiological Laboratory of the Animal Science Department of the College of Basic and Applied Sciences (CBAS) University of Ghana, Legon.

##### 3.1.1 Experimental Design

The experiment was a factorial design with sixteen treatments (control-clove, onion, white pepper, and nutmeg, African pepper (AP), African nutmeg (AN) and mixture of AP and AN) at 4 inclusion levels (0%, 0.05%, 0.1% and 0.15% of the meat weight) Appendix 4. There were three replicates. Products were evaluated for the effect of the spice mix on sensory attributes and microbiological load of fresh pork sausage.

#### 3.2. Preparation of African Nutmeg (AN) and African Pepper (AP)

African nutmeg and African pepper were obtained from the local market in Accra. The spices were cleaned and washed thoroughly under running tap water and sundried. The dried African nutmeg (AN) were cracked manually to extract the kernels. The kernels were blended and stored in Ziploc bags under a room temperature. The seeds of the African pepper (AP) were blended with the pods and stored into Ziploc bags under room temperature. Both spices were sieved with a fine net. The spices were irradiated with a dose of 10 KGY at the Ghana Atomic Energy Commission (GAEC) Irradiation Centre. The scientific name and origin of the selected local spices are presented in Appendix 5.

### **3.2.1 Sausage Preparation**

Fresh whole pork carcass was obtained from the Livestock and Poultry Research Center (LIPREC) University farm and stored under refrigeration conditions (4°C) overnight. The carcass was cut up and deboned and the sausages formulated, after using 80% lean and 20% fat. The meat was comminuted using a table-top mincer through 5mm steel plate (Model Talleres Ramon, Spain). The comminuted meat was manually mixed thoroughly in a mixing bowl. The mixture was divided into 16 equal batches by weight and treatment assigned to the batches randomly. The respective spice mixes and water (Appendix 4) were added and mixed manually (50 times). The sausage mixture was stuffed into a natural casing using manual stuffer and linked into 15cm length. The products were bagged into Ziploc bags and sealed labeled and kept under freezing temperature (-10°C) prior to sensory and microbial analysis.

### **3.2.2. Sensory Evaluation**

Ten panelists were screened for sensitivity to fishy and cardboard flavours using a nine two-fold dilutions of exudates from fresh tilapia and 10g of cardboard boiled in distilled water for 1 hr. Each set of flavour profile was provided with a distilled water control. The panelists were also screened for crumbliness and juiciness. Consequently, six panelists made up of 4 females and 2 males were short-listed to participate in the sensory evaluation. The samples were grilled (Electrical grill) separately based on treatments to an internal temperature of 71° C, for 45 minutes. The grilled sausages were sliced into uniform sizes approximately 15 g; randomly assigned labels and packed into respective coded 30ml containers and covered. The evaluation of the inclusion levels of the selected local spices was done by the use of a 15-point scale (Appendix 6) to evaluate crumbliness, juiciness, palatability, saltiness, off flavour and overall liking of the fresh

pork sausage. Water and apple juice were provided to rinse off lingering odours after every sample.

### **3.3 Plate Counts**

Sausage sample (1g) taken from the mid section was homogenized in 10 ml of distilled water using a blender. The homogenate was serially diluted ( $10^{-3}$  and  $10^{-4}$ ), plated on nutrient agar (aerobic plate count-APC) in triplicates and incubated at 37° C for 24 hours (Oluwafemi and Simisaye, 2006) to determine total viable counts. The same procedure was repeated for enterobacteriaceae count and *E. coli* / *Coliform* selective agar was used. The microbial assay were evaluated for respective treatments stored for day 0, 3 and 6 at 4°C for all the three replicates.

### **3.4 Statistical Analysis**

The microbial counts obtained from total bacteria, *E. coli* and coliform for each treatment was transformed to log cfu/g. The transformed values as well as results for sensory data were subjected to analysis of variance using proc mixed of SAS 9.1 (SAS Institute, Cary, NC, USA). SAS syntax for analysis (Appendix 7 and 8) are indicated. Probability differences between means were compared using bonferroni multiple comparison adjustment. Differences were considered significant at the  $p \leq 0.05$  level at 0.5.

## CHAPTER FOUR

## RESULTS

## 4.1. Sensory Evaluation

Results of evaluated fresh pork sausages treated with varying levels of AP and AN on a 15-point scale for crumbliness, juiciness, palatability, saltiness, off flavour and overall liking. Results for crumbliness and juiciness are shown in Table 1. Inclusion levels of AN (0.05 %), and AP\*AN (0.05\*0.05%)

Table 1. Mean Crumbliness and Juiciness

Treatment	Crumbliness		Juiciness	
	Mean	SEM	Mean	SEM
Control	8.2	±0.6	6.6	±0.74
AN (0.05%)	8.9	±0.6	6.2	±0.74
AN (0.1%)	8.3	±0.61	6.4	±0.74
AN (0.15%)	8.4	±0.6	6.6	±0.74
AP (0.05%)	8.6	±0.59	6.2	±0.74
AP (0.1%)	8.4	±0.59	6.6	±0.74
AP (0.15%)	8.7	±0.59	6.3	±0.74
AP*AN (0.05*0.05%)	8.9	±0.6	6.9	±0.74
AP*AN (0.05*0.1%)	8.7	±0.59	6.8	±0.74
AP*AN (0.05*0.15%)	7.9	±0.59	6.7	±0.74
AP*AN (0.1*0.05%)	7.9	±0.6	7.6	±0.74
AP*AN (0.1*0.1%)	8.0	±0.59	6.8	±0.74
AP*AN (0.1*0.15%)	8.6	±0.6	7.6	±0.74
AP*AN (0.15*0.05%)	8.1	±0.59	6.7	±0.74
AP*AN (0.15*0.1%)	8.4	±0.6	5.9	±0.74
AP*AN (0.15*0.15%)	7.8	±0.59	6.1	±0.74
P-value	0.62		0.47	

SEM, standard error of mean

AP; African pepper, AN; African nutmeg, AP\*AN; combination of AP and AN

Levels of inclusion (%) are indicated in parenthesis had the highest scores (8.9), for crumbliness and AP\*AN (0.15\*0.15%) had the lowest scores (7.8) as compared to the control product (8.2). Statistical analysis of the data indicated no significant ( $p \geq 0.05$ ) differences between any of the treatments. Although mean scores indicated that AP\*AN (0.1\*0.05%) and AP\*AN (0.1\*0.15%) had the highest scores value (7.6) for juiciness and AP\*AN (0.15\*0.1%) score the lowest (5.9) as compared to the control (6.6), statistical analysis of the data indicated no significant ( $p \geq 0.05$ ) differences between any of the treatments compared to the control. Hence sausages formulated with the selected local spices at varying concentrations did not differ ( $p \geq 0.05$ ) from the control product in terms of terms of juiciness.

The results for palatability and saltiness are shown in Table 2. Mean scores indicated that although AP treated sausage at 0.15% and AP\*AN (0.1\*0.1%) inclusion levels had the highest scores (9.1) for palatability and AN (0.1%) and AP\*AN (0.05\*0.1%) had the lowest scores (7.9) as compared to control product (8.1). Statistical analysis of the data however indicated no significant ( $p \geq 0.05$ ) differences between any of the treatments compared to the control. Mean scores for saltiness indicated that AP\*AN (0.05\*0.1%) treated sausage had the highest scores (8.3) and AN (0.15%) scored the lowest (6.5) as compared to the control product (6.6). Statistical analysis of the data showed no significant ( $p \geq 0.05$ ) differences between any of the treatments compared to the

**Table 2. Mean Palatability and Saltiness**

Treatment	Palatability		Saltiness	
	Mean	SEM	Mean	SEM
Control	8.1	±1.01	6.6	±0.59
AN (0.05%)	8.4	±1.01	7.6	±0.6
AN (0.1%)	7.9	±1.01	7.2	±0.59
AN (0.15%)	8.1	±1.01	6.5	±0.59
AP (0.05%)	8.1	±1.01	7.1	±0.59
AP (0.1%)	8.7	±1.01	7.4	±0.59
AP (0.15%)	9.1	±1.01	6.9	±0.59
AP*AN (0.05*0.05)	8.5	±1.01	7.1	±0.59
AP+AN (0.05/0.1%)	7.9	±1.01	8.3	±0.59
AP*AN (0.05*0.15%)	8.2	±1.01	7.0	±0.59
AP*AN (0.1*0.05%)	8.9	±1.01	7.3	±0.6
AP*AN (0.1*0.1%)	9.1	±1.01	7.7	±0.6
AP*AN (0.1*0.15%)	8.1	±1.01	7.1	±0.59
AP*AN (0.15*0.05%)	8.9	±1.01	7.1	±0.59
AP*AN (0.15*0.1%)	9.0	±1.01	7.5	±0.59
AP*AN (0.15*0.15%)	8.4	±1.01	6.8	±0.59
P-value	0.15		0.21	

SEM, standard error of mean.

AP; African pepper, AN; African nutmeg, AP\*AN; combination of AP and AN

Levels of inclusion (%) are indicated in parenthesis.

control. Evaluation of the off flavour and overall liking of the samples at varying concentrations of the local spices are shown in Table 3.

**Table 3. Mean Off-Flavour and Overall-Liking**

Treatment	Off flavour		Overall liking	
	Mean	SEM	Mean	SEM
Control	4.2	±1.17	8.8	±0.95
AN (0.05%)	4.7	±1.19	8.9	±0.97
AN (0.1%)	3.7	±1.18	8.3	±0.95
AN (0.15%)	4.3	±1.18	8.5	±0.97
AP (0.05%)	4.5	±1.19	8.1	±0.95
AP (0.1%)	4.1	±1.20	8.7	±0.95
AP (0.15%)	4.6	±1.21	9.1	±0.95
AP*AN (0.05*0.05)	4.0	±1.21	8.1	±0.95
AP*AN (0.05*0.1%)	4.1	±1.20	8.3	±0.95
AP*AN (0.05*0.15%)	3.5	±1.20	8.0	±0.95
AP*AN (0.1*0.05%)	4.4	±1.19	8.8	±0.95
AP*AN (0.1*0.1%)	4.7	±1.18	8.9	±0.95
AP*AN (0.1*0.15%)	3.9	±1.20	8.8	±0.95
AP*AN (0.15*0.05%)	3.9	±1.21	9.1	±0.95
AP*AN (0.15*0.1%)	3.7	±1.19	9.3	±0.95
AP*AN (0.15*0.15%)	4.1	±1.19	9.2	±0.96
P-value	0.96		0.62	

SEM, standard error of mean.

AP; African pepper, AN; African nutmeg, AP\*AN; combination of AP and AN. Levels of inclusion (%) are indicated in parenthesis.

Mean scores for off flavour showed that AN at 0.05% and AP\*AN at 0.1\*0.1% inclusion levels had the highest scores (4.7), while AP\*AN at 0.05\*0.15% had the lowest score (3.5) as compared to the control product (4.2). Statistical analysis of the data showed no significant ( $p \leq 0.05$ ) differences between any of the treatments compared to the control. Mean scores for overall liking indicated that although AP\*AN at 0.15\*0.1% inclusion level had the highest score (9.3), and between the two individual local spice treatments, sample treated with AP at 0.15 % concentration had a higher score (9.1) and AN at 0.05% concentration had (8.9) among its inclusion levels. AP treated sample at 0.05% and AP\*AN at 0.05\*0.05% inclusion levels scores the lowest (8.1) as compared to the control products (8.8), and statistical analysis of the data indicated no significant ( $p \leq 0.05$ ) differences between any of the treatments.

#### **4.2. Microbial Analysis**

Aerobic bacteria, coliforms and *E. coli* were counted for the respective treatments stored for 0, 3, and 6 days at 4°C. (Table 4). Compared to the control, substituting AN with nutmeg up to 0.15% significantly increased aerobic bacteria count. However, the substitution did not affect coliform and *E. coli* counts.

**Table 4. (Log<sub>10</sub> CFU/g) Count of Aerobic Bacteria, Coliforms and *E. coli***

Treatment	APC	SEM	Coliform	SEM	E coli	SEM
Control	6.93 <sup>a</sup>	±0.33	6.55	±0.17	3.01	±0.53
AN (0.05%)	7.19 <sup>c</sup>	±0.33	6.67	±0.17	2.55	±0.53
AN (0.1%)	7.22 <sup>c</sup>	±0.33	6.70	±0.17	3.51	±0.53
AN (0.15%)	7.09 <sup>b</sup>	±0.33	6.67	±0.17	2.50	±0.53
AP (0.05%)	6.94 <sup>a</sup>	±0.33	6.45	±0.17	1.67	±0.53
AP (0.1%)	7.01 <sup>a</sup>	±0.33	6.53	±0.17	2.53	±0.53
AP (0.15%)	6.95 <sup>a</sup>	±0.33	6.30	±0.17	0.85	±0.53
AP*AN (0.05*0.05)	7.12 <sup>b</sup>	±0.33	6.64	±0.17	2.64	±0.53
AP*AN (0.05*0.1%)	6.94 <sup>a</sup>	±0.33	6.47	±0.17	2.18	±0.53
AP*AN (0.05*/0.15%)	6.93 <sup>a</sup>	±0.33	6.35	±0.17	1.26	±0.53
AP*AN (0.1*0.05%)	7.06 <sup>a</sup>	±0.33	6.45	±0.17	1.82	±0.53
AP*AN (0.1*0.1%)	7.04 <sup>a</sup>	±0.33	5.80	±0.17	1.64	±0.53
AP*AN (0.1*0.15%)	7.02 <sup>a</sup>	±0.33	6.51	±0.17	2.5	±0.53
AP*AN (0.15*0.05%)	7.01 <sup>a</sup>	±0.33	6.55	±0.17	2.08	±0.53
AP*AN (0.15*0.1%)	6.98 <sup>a</sup>	±0.33	6.65	±0.17	2.05	±0.53
AP*AN (0.15*0.15%)	6.97 <sup>a</sup>	±0.33	6.68	±0.17	2.53	±0.53
P-value	0.62		0.62		0.62	

AP; African pepper, AN; African nutmeg, AP\*AN; combination of AP and AN

APC; Aerobic plate count, SEM: Standard error of mean

Means with the same superscripts within a column are not significantly ( $p \leq 0.05$ ) different.

Furthermore, substituting white pepper with AP did not affect aerobic bacteria, count and none of the combinatorial substitutions affected aerobic bacteria, coliform and *E. coli* counts except for an observed increase in APC count for substituted AP\*AN (0.05\*0.05) (Table 4).

Results for microbial count during storage at 0, 3 and 6 d at 4°C are shown in Table 5. For APC, mean log<sub>10</sub> CFU/g count for aerobic bacteria and coliforms significantly ( $p \leq 0.05$ ) increased between day 0 and day 3 and decreased from day 3 to 6.

**Table 5. Log<sub>10</sub> CFU/g Count of Aerobic Bacteria, Coliforms and *E. coli* During Storage**

Days	APC	SEM	Coliform	SEM	<i>E. coli</i>	SEM
0	6.97 <sup>b</sup>	±0.02	6.25 <sup>a</sup>	±0.07	2.10 <sup>b</sup>	±0.25
3	7.24 <sup>c</sup>	±0.02	6.69 <sup>b</sup>	±0.07	1.34 <sup>a</sup>	±0.25
6	6.87 <sup>a</sup>	±0.02	6.56 <sup>b</sup>	±0.07	2.99 <sup>c</sup>	±0.25

APC: Aerobic plate count

SEM: Standard error of mean

Means with the same superscripts within a column are not significantly ( $p \leq 0.05$ ) different.

For coliform, mean log count similarly increased from day 0 to day 3. No significant ( $p \leq 0.05$ ) difference in log count was observed from day 3 to day 6. Lastly, for *E. coli* log<sub>10</sub> CFU/g count decreased from day 0 to day 3, however, a significant increase in log count was observed from day 3 to day 6.

## CHAPTER FIVE

### DISCUSSION

#### 5.1. Sensory Evaluation

Crumbliness, texture and flavour are important criteria for evaluating eating quality of meat and meat products. Methods of cooking of meat products can affect some characteristics of meat products such as crumbliness and juiciness (FAO, 1999). In this study, panelists evaluated fresh pork sausage, substituted with two local spices at varying inclusion levels (between 0%-.15%) using a factorial design. White pepper was substituted with African pepper (*Xylopiya aethiopica*) and nutmeg was substituted with African nutmeg (*Monodora myristica*). Sensory attributes evaluated were crumbliness, juiciness, palatability, saltiness, off-flavor and overall acceptability. Statistical analysis showed no significant ( $p \geq 0.05$ ) differences among treatments with substituted local spices compared to the control on all the sensory attributes measured.

The historical use of spices and the long established recipes for sausage production and the difficulty of objective evaluation of combination effects of spices on flavour has resulted in a dearth of knowledge on the effects of spices on sensory attributes. Aidells and Kelly (1998) indicated that spices improve texture, juiciness and flavour of meat and meat product. Juiciness is one of the most important sensory quality attributes when evaluating consumer liking (Resurreccion, 2004). A study conducted by Abisoye *et al.* (2013) reported that plants phenolic compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma, juiciness and flavour, it also provides health beneficial effects. The low juiciness scores might be due to loss of moisture from the products during the storage time (1 day) and during the grilling. The results were in accordance with findings of Zargar *et al.* (2014) who reported decreasing trend in

chicken sausages as results of loss of moisture from the products during storage. Similarly, Jay (1996) also reported that reduction in pH and denaturation of protein at low pH and degradation of muscle fibre protein by bacterial action can lead to a decrease in juiciness of sausage. Juiciness and tenderness decreased with an increasing temperature from 62- 75 °C, while crumbliness and chewing time increases (FAO, 1999). To reduce the variance in sensory attributes due to changes in cooking temperature in this study, all treatments were grilled to an internal temperature of 71 °C. All the treatments were acceptable by the trained panelists.

The quality of meat and meat product is defined by its palatability and this can be achieved by organoleptic evaluation. The higher score for the AP treated sausage at 0.15% (Table 3) was probably due to the fact that the panelists are used to its distinctive flavour and aroma in Ghanaian dishes. The present study reveals that the two-spice mixture (0.1\*0.1%) was rated higher as compared with the control, which agreed with findings of Sodzim (2012). The essential oil contents in spices are noted for flavour and taste enhancer (Ekanem and Achimewhu, 1998).

Spices are not only used for flavouring food but are also used to enhance latent flavour of food (Hui *et al.*, 2005). The results from the study showed that all the treatments were within the same range. According to the study conducted by Hui *et al.* (2005), spices have a low sodium contents that can be used as salt substitute. The present study reveals the correlation between spice and salt in meat products. Carraro *et al.* (2012) reported that the addition of spices and herbs in a reduced sodium content of bologna sausage resulted in better sensory attributes. Although saltiness was tested under the hypothesis

that the substitute spices may potentiate the perception of salt, no significant differences in saltiness were observed between any of the treatments compared to the control.

Flavour comprises mainly of taste and aroma and is an indicator of consumers' purchase intent and preferences. The sensory scores of off flavour were not statistically different among the treatment means. Flavour can be developed during processing, cooking and through spices and complex reactions (Dinesh *et al.*, 2013). Many compounds that contributed to the overall odour of fresh sausages have been identified as spiciness caused by phenols and terpens in spices.

The results from the present study observed AP treated sausages had an increasing trend of overall liking as the inclusion levels increases. Sensory analysis revealed that the overall liking is directly proportional to the concentration of the spices used. Addition of spices may cause some changes in the meaty flavour of fresh pork sausages; however, these changes were acceptable to the panelists. Thus the present study revealed that AP and AN can be used to substitute for white pepper and nutmeg without affecting the sensory characteristics of fresh pork sausages.

## 5.2. Microbial Count

The observed increased counts of APC during the storage period from 0 to 3 d ( $7.0 - 7.6 \log_{10}$  CFU/g) was above the maximum recommended limit ( $7 \log_{10}$  CFU/g) set by (ICMSF, 1986) for APC in processed meat. Marie-Josee *et al.*, (2001) documented antimicrobial effect of mustard extracts on microbes on chicken meat. According to Bahk *et al.*, (1990), plant extracts might extend the lag phase, the generation time and decreases the growth rate of microbes.

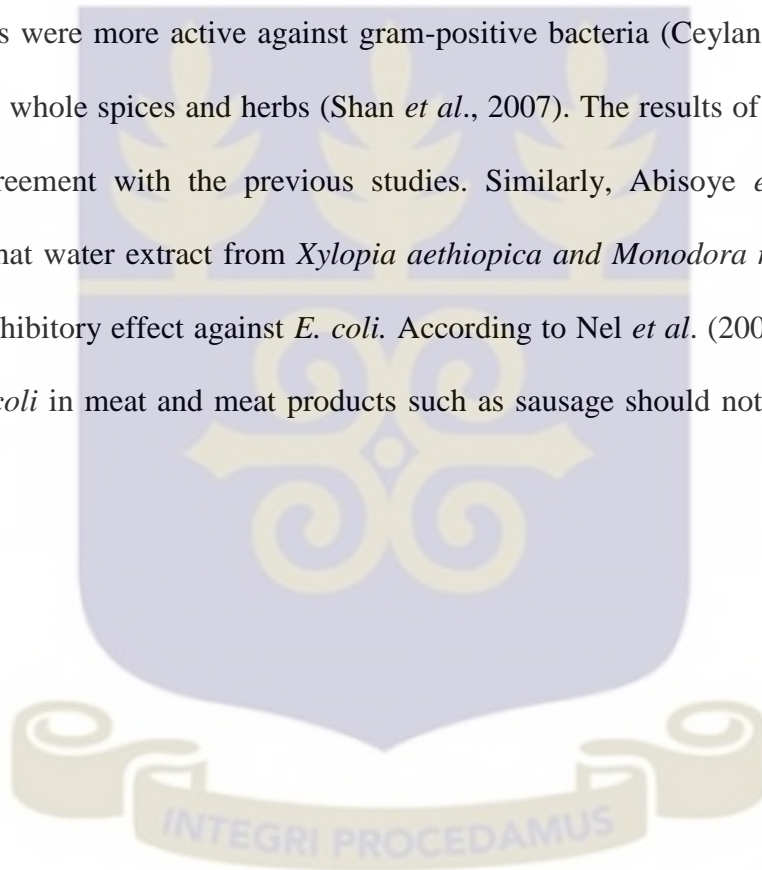
A number of studies (Lambert *et al.*, 2001; Davidson and Naidu, 2000) have suggested that plant extracts that possess very strong antimicrobial properties against pathogens contain a high percentage of phenolic compounds. From our results as presented in Table 4, contrary to the concept of antimicrobial properties of spices as mustard (Marie-Josee *et al.*, 2001) our investigations showed no antimicrobial properties of AP and AN at the concentrations ( $\leq 0.1\%$ ) tested. On the contrary, substituting nutmeg (Table 4) with AN increased APC count significantly ( $p < 0.05$ ) compared to the control. This could be result of possible contamination as suggested by Sachindra *et al.* (2005) that the microbial composition of meat and meat products might depend mainly on the kind of environment, the type of meat and raw materials, equipment, packaging materials, from the water and storage temperature.

It was also interesting to note that AP\*AN (0.05\*0.05) elicited increased log counts of aerobic bacteria. Although these observations may have resulted from residual bacteria in the AN preparation used, further studies may be needed to elucidate the background microbial counts of the spices used. Groups of bacteria studied in this experiment appear to have exhibited different growth patterns during storage. While APC count increased from day 0 to day 3, it decreased from day 3 to day 6. This growth model suggests a possible introduction of that negative feedback factors that reduced the population of aerobic bacteria.

The growth pattern displayed by aerobic bacteria could have resulted from antagonistic activity due to the decrease in pH and increase competition for available substrate (Russo *et al.*, 2006). Organisms of high metabolic activity may consume the selective required nutrients and inhibit other organisms. Similarly, (ICMF, 1980) reported that *Streptococci*

inhibit growth of *Staphylococci* by exhausting the supply of nicotinamide or niacin and biotin. It is believed that lag period and growth rate of aerobic bacteria might have been influenced by combined impact of temperature, pH and  $a_w$  as well as the antimicrobial activity of the two selected spices, as reported by (Jay, 2000; FDA, 2001).

Evidently, negative feedback factors cannot explain growth trends observed for coliforms and *Escherichia coli* counts over the 6 day storage period. Thus, growth patterns of the groups of bacteria assayed were different. It has been established that essential oils were more active against gram-positive bacteria (Ceylan and Fung, 2004) so are many whole spices and herbs (Shan *et al.*, 2007). The results of the present study were in agreement with the previous studies. Similarly, Abisoye *et al.* (2013) has concluded that water extract from *Xylopiya aethiopica* and *Monodora myristica*, did not show any inhibitory effect against *E. coli*. According to Nel *et al.* (2004), the maximum limit of *E. coli* in meat and meat products such as sausage should not be more than 10 cfu/g.



## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

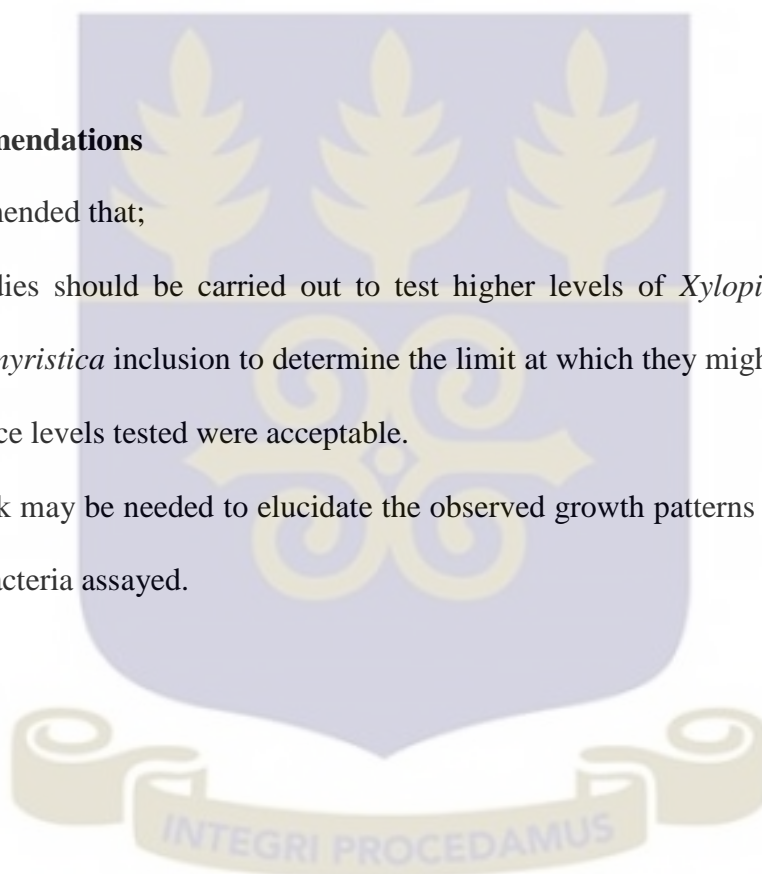
The present study has shown that *Xylopiya aethiopica* and *Monodora myristica* can be used to substitute for *Piper nigrum* and *Myritica fragrans* respectively in the manufacture of fresh pork sausages without affecting the sensory attributes and overall liking of the product.

#### 6.2 Recommendations

It is recommended that;

Further studies should be carried out to test higher levels of *Xylopiya aethiopica* and *Monodora myristica* inclusion to determine the limit at which they might affect a sensory attribute since levels tested were acceptable.

Further work may be needed to elucidate the observed growth patterns for the respective groups of bacteria assayed.



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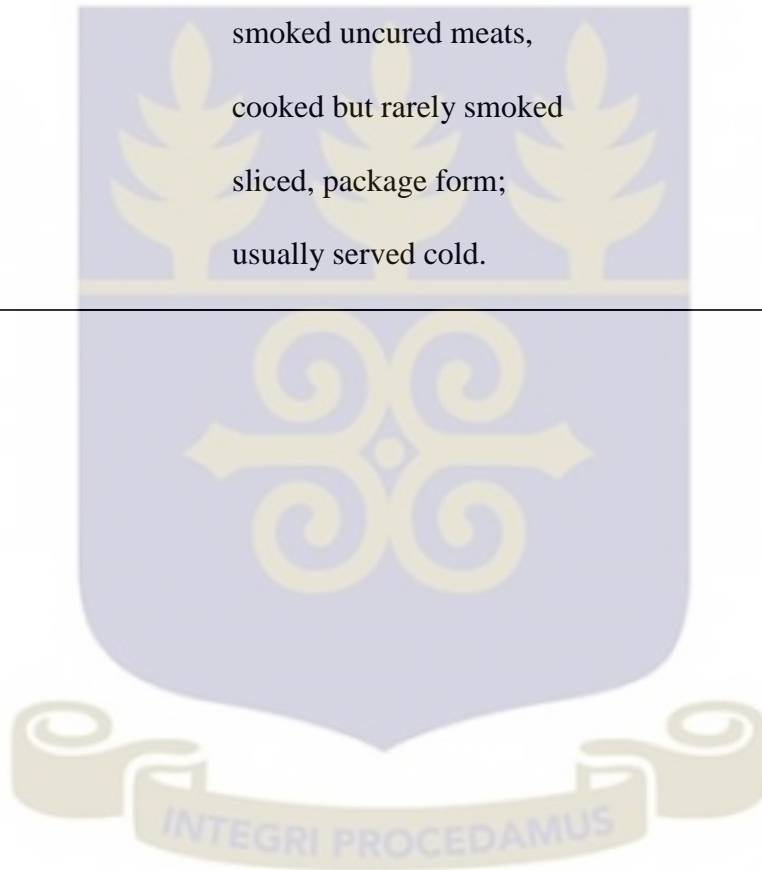
## APPENDICES

### Appendix 1: Sausage Classification

Classification	Characteristics	Examples
Fresh sausage	Fresh meat (mainly pork) uncured, comminuted, seasoned and usually stuffed into casing, must be cooked before serving.	Fresh pork sausage, Bratwurst, Boerewore
Dry and semidry sausages	Cured meat, fermented air dried, may be smoked before drying; served cold.	Gonoa salami, Ppperoni Len bologna Summer sausage
Cooked sausage	Cured or uncured meat; comminuted seasoned, stuffed into casings, cooked and sometimes smoked; served cold.	Liver sausage Braunscheweiger
Smoked sausages	Cured meats; communitied, seasoned, stuffed into casings smoked and fully cooked; do not require further cooking, but some are heated.	Frankfurters, Bologna, Cotto salami

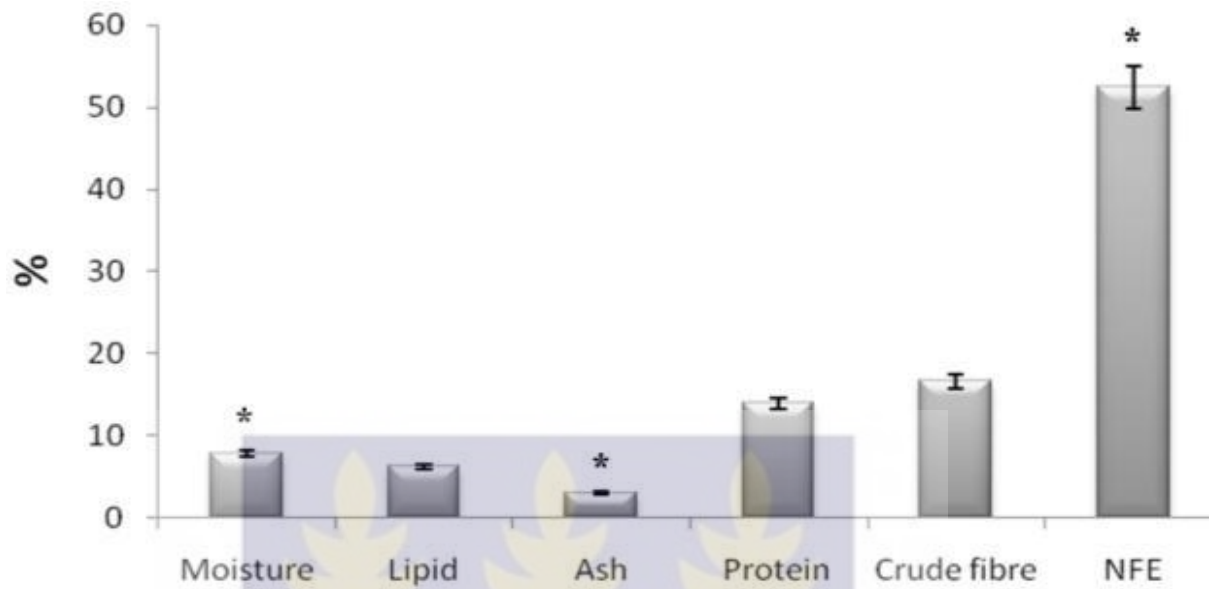
Uncooked, smoked sausage	Fresh meats; cured or uncred, stuffed smoked, but not cooked; must be fully cooked before serving.	Smoked, country-style pork sausage Mettwurst, Kielbasa
Cooked meat specialties	Specially prepared meat products; cured or uncured meats, cooked but rarely	Loaves, Headcheese, Scrapple

smoked uncured meats, cooked but rarely smoked sliced, package form; usually served cold.



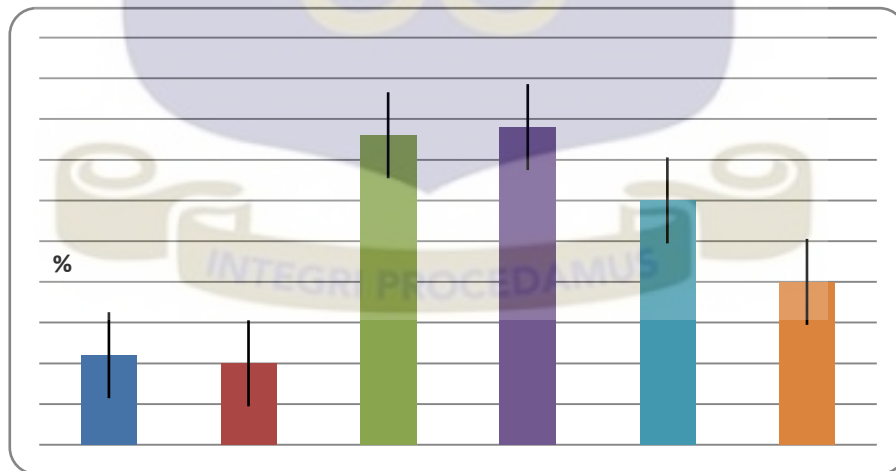
**Appendix 2: Classification of Natural Spices Commonly Found in Ghana**

<b>Spice Common name</b>	<b>Botanical name</b>	<b>Family name</b>	<b>Locacal name</b>	<b>Origin</b>	<b>Part use</b>
Black & white pepper	<i>Piper nigrum</i>	<i>Piperaceae</i>	Kalen	India	Seed
African pepper	<i>Xylopi aethiopica</i>	<i>Annonaceace</i>	Etso, Hwentea	West Africa	Fruit/seed
African nutmeg	<i>Monodora myristica</i>	<i>Annonaceace</i>	Ayiku, Awiriwa	West Africa	Seed
Nutmeg	<i>Myristica fragrans</i>	<i>Myristicaceae</i>		Indonesia	Seed
Cloves	<i>Syzygium aromaticum</i>	<i>Myrtaceae</i>	Pepree	Indonesia	Flower bud
Mace	<i>Myristica fragrans</i>	<i>Myristicaceae</i>		Indonesia	Flower
Sweet Basil	<i>Ocimum basilicum</i>	<i>Labiataeae</i>	Akokobesa	India	Leaves
Marjoram	<i>Origanum majorana</i>	<i>Lamiaceae</i>		Cyprus	Leaves
Onion	<i>Allium cepa</i>	<i>Amaryllidaceae</i>	Sabolai, Gyeene	Egypt	Bulb
Garlic	<i>Allium sativum</i>	<i>Amaryllidaceae</i>	Ayo	Asia	Bulb
Ginger	<i>Zingiber officinale</i>	<i>Zingiberaceae</i>	Kakadro	Asia	Rhizome/Root
Cinnamon	<i>Cinnamomum verum</i>	<i>Lauraceae</i>		Sri Lanka	Bark



**Figure 1: Proximate Composition of *Monodora myristica Xylopi aethiopica*.**

Values are mean  $\pm$  SD (n = 3); \*p < 0.05. (Ochuko *et al.*, 2012)



**Figure 2: Proximate Composition of *Xylopi aethiopica***

Values are mean  $\pm$  SD

**Appendix 3: Phytochemicals Properties of *Monodora myristica***

<b>/Phytochemicals (g/100)</b>	<b>Screening</b>	<b>% Quantity</b>
Tannin	+	1.67 + 0.17
Cardiac glycosides	+	NQ
Cardiac glycosides	+	NQ
Steroids	+	NQ
Terpenoids	+	NQ
Saponin	+	4.27 + 0.15
Alkaloids	+	0.51 + 0.25
Phlobatannins	-	-

**Key:** + = present; - = absent; NQ = not quantified (Ochuko *et al.*, 2012).

**Phytochemicals Properties of *Xylopiya aethiopica***

<b>Phytochemicals (g/100)</b>	<b>Screening</b>
Tannin	+
Glycosides	+
Steroids	+
Terpenoids	+
Saponin	+
Alkaloids	+
Reducing sugar	+
Flavonoids	+
Phynol	+

(+) present/detected

**Appendix 4: Formulations**

Ingredient, Gram	Treatment															
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16
Pork shoulder meat	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8	550.8
Water	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88	112.88
Salt	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16	8.16
Dextrose/Sucrose	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
Cloves	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Onion	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
African Pepper	0	0	0	0	0.34	0.68	1.02	0.34	0.34	0.34	0.68	0.68	0.68	1.02	1.02	1.02
African nutmeg	0	0.34	0.68	1.02	0	0	0	0.34	0.68	1.02	0.34	0.68	1.02	0.34	0.68	1.02
White pepper	0.34	0.34	0.68	1.02	0	0	0	0	0	0	0	0	0	0	0	0
Nutmeg	0.34	0	0	0	0.34	0.68	1.02	0	0	0	0	0	0	0	0	0

T1=Control, T2=AN (0.05%), T3=AN (0.1%), T4=AN (0.15%), T5=AP (0.05%), T6 =AP (0.1%), T7=AP (0.15%), T8= AP\*AN (0.05\*0.05%), T9=AP\*AN (0.05\*0.1%), T10=AP\*AN (0.05\*0.15%), T11=AP\*AN (0.1\*0.5%), T12= AP\*AN (0.1\*0.1%), T13= AP\*AN (0.1\*0.15%), T14=AP\*AN (0.15\*0.05%), T15=AP\*AN (0.15\*0.1%) and T16= AP\*AN (0.15\*0.15%).

**Appendix 5: Common Name, Scientific and Origins of *Xylopi*a *aethi*o*pica* and**

***Monodora myristica***

<b>Common Name</b>	<b>Botanical Name</b>	<b>Local Name</b>	<b>Family Name</b>	<b>Origin</b>	<b>Part Used</b>
African pepper	<i>Xylopi</i> a <i>aethi</i> o <i>pica</i>	<i>Etso</i> “ “ <i>Hwentea</i>	<i>Annonaceae</i>	West Africa	Fruits
African nutmeg	<i>Monodora</i> <i>myristica</i>	<i>Awiriwa</i> ” “ <i>Eyuku</i>	<i>Annonaceae</i>	West Africa	Fruits & seeds

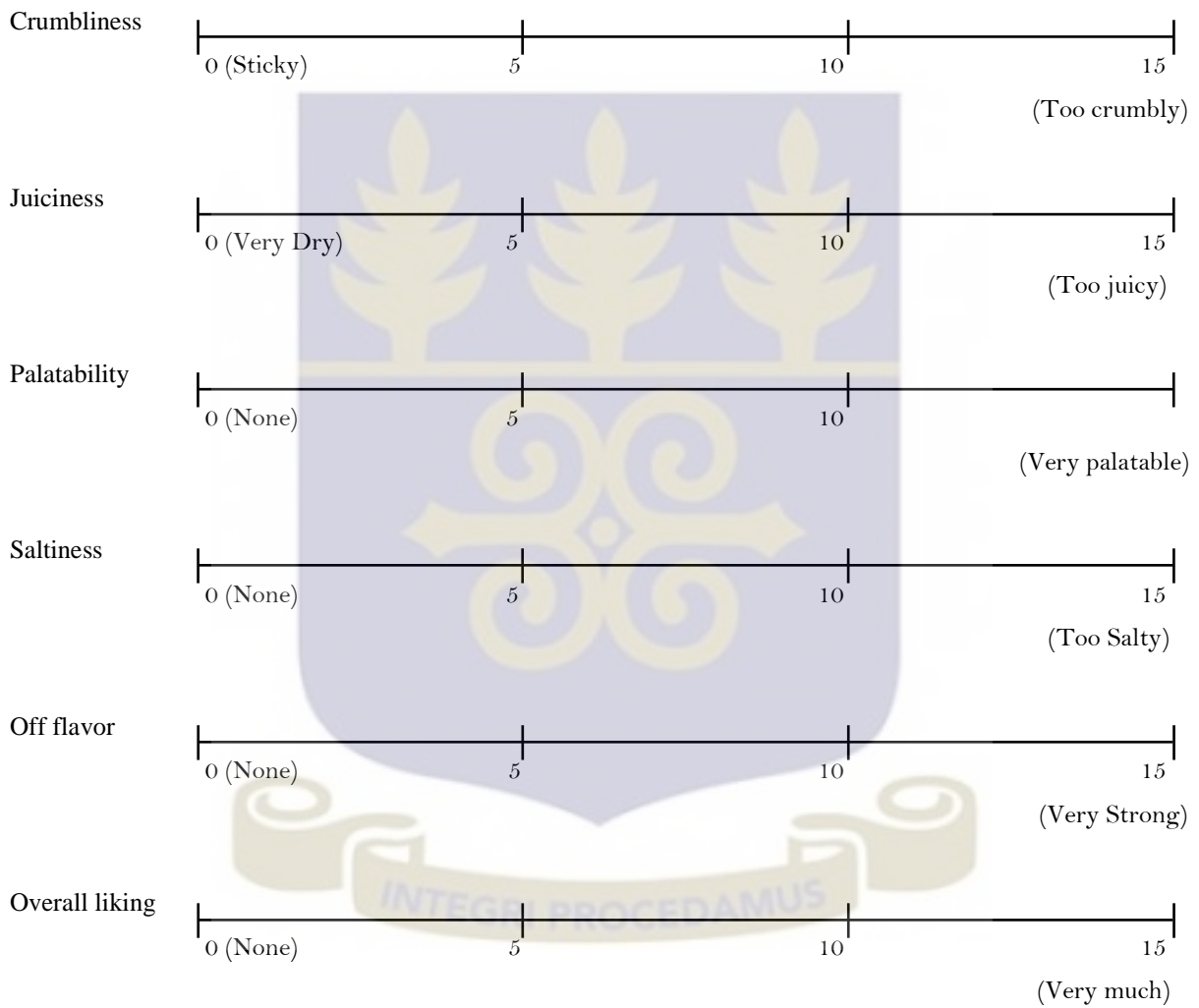


### Appendix 6: Meat Lab - Evaluation Sheet

Department of Animal Sciences, College of Agriculture and Consumer Sciences

#### Meat Lab - Evaluation Sheet

Sample ID ..... Initials .....





**Plate 1: Unripe (fresh) fruits of *Xylopiya aethiopica***





**Plate 2: Dried fruits of *Xylopiya aethiopyca***





**Plate 3: Dried nuts of *Monodora myristica***

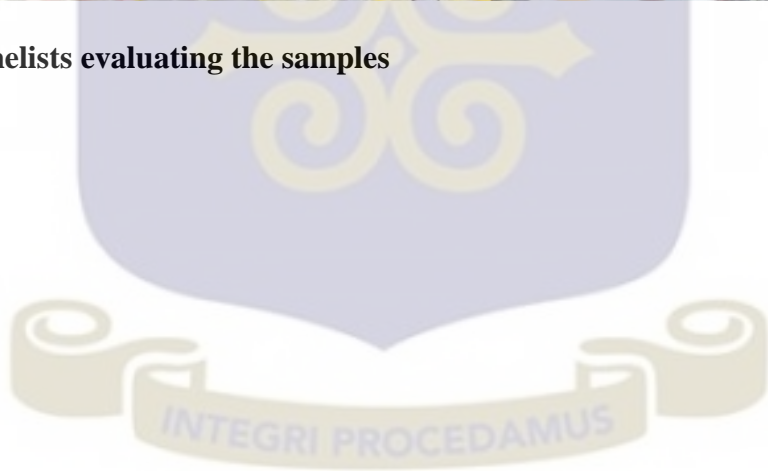


**Plate 4: Tote boxes to transport samples for irradiation**



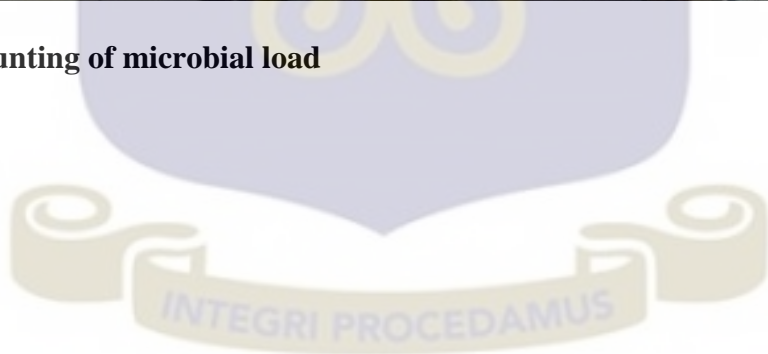


**Plate 5: Panelists evaluating the samples**



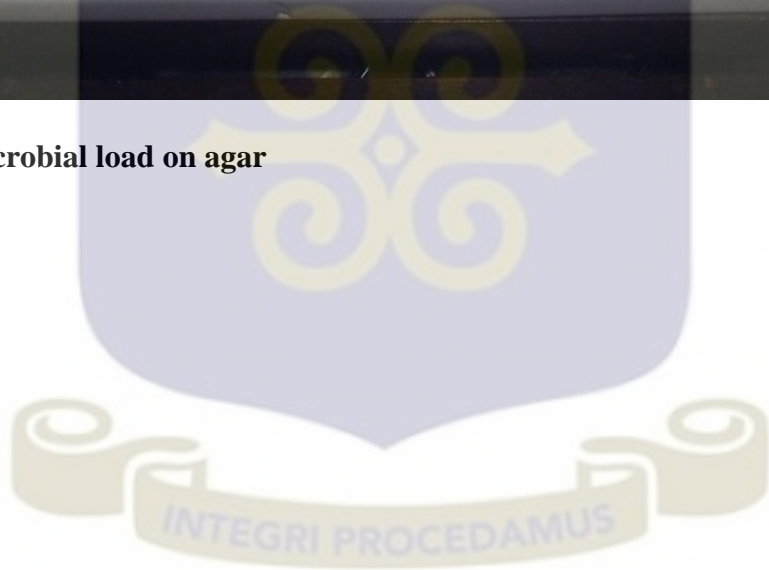


**Plate 6: Counting of microbial load**





**Plate 7: Microbial load on agar**



## Appendix 7: SAS Syntax for Sensory Data

```
options ls = 100 ps = 100 nocenter;  
data sensory;  
input Pan $ Sex $ Rep Trt Crumb Juic Pala Salt Flav OverL @@;  
datalines;
```

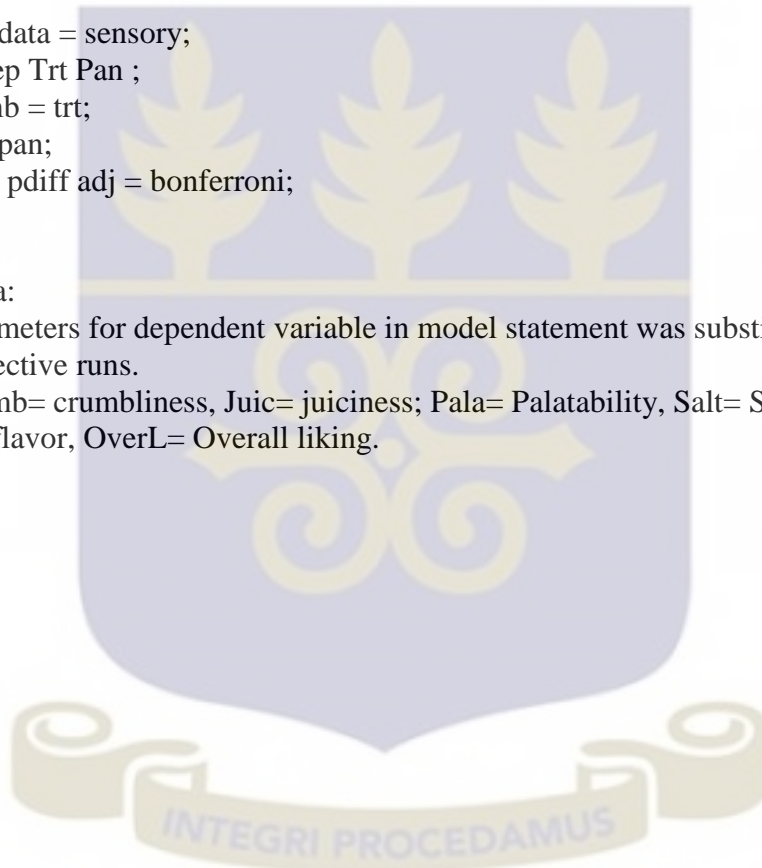
”

```
proc univariate plot normal plot data =sensory;  
var Crumb /*Crumb Juic Pala Salt Flav OverL*/;  
run;
```

```
Proc mixed data = sensory;  
class Sex Rep Trt Pan ;  
model Crumb = trt;  
random rep pan;  
lsmeans trt / pdiff adj = bonferroni;  
run;
```

Sensory data:

1. Parameters for dependent variable in model statement was substituted for respective runs.
2. Crumb= crumbliness, Juic= juiciness; Pala= Palatability, Salt= Saltiness, Flav= Off flavor, OverL= Overall liking.



### Appendix 8: SAS Syntax for Bacteria log Count Data

```
options ls = 120 ps = 120 nocenter;
data Micro;
input Trt      Rep Day      Org $ Rep Count VPlate Dilut VBlend count Log @@ ;
datalines;

”

Proc sort data = Micro;
by org ;
run;

Proc mixed data = Micro;
class Rep Day Trt ;
model log = trt|day;
by org ;
random rep;
lsmeans trt|day /pdiff adjust=bon;
run;
```

