KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

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DEPARTMENT OF ANIMAL SCIENCE

THE PRESERVATIVE EFFECTS OF NATURAL HERBS/SPICES USED IN MEAT PRODUCTS

BY

YAMOAH ANTHONY GIDEON

(B.Sc. Agriculture (Animal Science), Hons)

A THESIS SUBMITTED IN PARTIAL FUFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY IN MEAT SCIENCE

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A Thesis

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in

Partial Fulfillment of the Requirement for the Degree of

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DECLARATION

I hereby declare that this research was carried out by me and that this thesis is entirely my own account of the research. The work has not been submitted to any other University for a degree.

However, works of other researchers and authors which served as sources of information were duly acknowledged.

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(Meat Science /Food Safety) Signature Date

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Certified by:

Mr. WORLAH YAWO AKWETEY ………………..... ………………..

(Head of Department) Signature Date

DEDICATION

I dedicate this work to my mother- Perpetual Annobil, my wife - Mrs. Naomi A. Yamoah and my son - Kant Owusu Ansah.

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LISTS OF ABBREVIATIONS

CFU	-	Colony Forming Unit
Cp	-	Chili Pepper
CpE	-	Chili Pepper Essential Oil
DPPH	-	1, 1- Diphenyl-2-picrylhydrazyl
E. coli	-	*Escherichia coli*
FFA	-	Free Fatty Acids
GE	-	Ginger Essential Oil
NP	-	Negro Pepper
NPE	-	Negro Pepper Essential Oil
OAC	-	Oil Absorption Capacity
OE	-	Onion Essential Oil
PRE	-	Prekese Essential Oil
SD	-	Standard Deviation
Staph	-	*Staphylococcus*
TPC	-	Total Plate Count
WAC	-	Water Absorption Capacity
EHEC	-	Enterohaemorrhagic E. coli

ABSTRACT

Sausage production and consumption is gaining prominence daily in Ghana. However, with the erratic power supply, products deterioration in terms of microbial and oxidative spoilage is on the increase. The study was therefore undertaken to curb this sitaution, hence, the objectives of this study were to assess the use of spices/herbs in meat/food preparation in the Kumasi Metropolis and evaluate the possibility of utilizing the essential oils of the spices/herbs as natural antioxidants and antimicrobials. A structured questionnaire was used to assess the utilization of spices and herbs in the study area. Based on the outcome of the questionnaire, spices/herbs of prominence (ginger, onion, chili pepper, prekese and negro pepper) were selected and their phytochemicals and functional properties analyzed using standard method. Essential oils extracted from these herb/spices were included in frankfurter - type sausage. The products were evaluated for physico-chemical properties and keeping qualities (microbial count and oxidative stabilities) using standard procedure. Overall acceptability was determined with 9-point hedonic scale. Data were analyzed using descriptive statistics and ANOVA at $p \leq 0.05$. The study showed that more females (75%) consume/use spice/herbs for domestic purposes than their male (25%) counterpart in meat/food preparation. Most of the respondents were Christian youth (26-35 yrs), married with household size less than four and Akan by tribe. Most of them had formal education up to secondary/tertiary level and engaged in business activities. Spices/herbs in powder or whole form were mostly used to make stew, soup, kebabs, sausages and burgers. Similar trend was observed for processors/vendors of meat products who mostly used the spices/herbs to prepare kebabs, sausages and burgers for their customers at an average cost of GH¢11.00 to

GH¢15.00 to produce a kg of meat product of which GH¢1.00 - GH¢5.00 was spent on spices. More males were involved in the processing/sale of meat products than males who consumed or used spices/herbs for domestic purposes. Ginger, onion, chili pepper, Negro pepper and prekese were the commonly used spices/herb in culinary activities in the study area. Phytochemicals (phenols, flavonoids, tannins, alkaloids and saponins) were pesent in the spices/herbs. Essential oil (0.05%) from each spice/herb was added in the manufacture of frankfurter sausages and the cooked product stored at -18 for three weeks. Proximate composition, free fatty acids (FFA), pH, 1, 1-Diphenyl-2picrylhydrazyl (DPPH) free radical scavenging assay, microbial load and organoleptic qualities were determined during storage. The study indicated that there were significant ($p<0.05$) increases in the FFA of all samples during day 7 and day 21 whilst day 14 recorded no significant ($p>0.05$) increase. In all, the ginger-treated products recorded the least FFA production at the end of storage. Antioxidant activity of prekese, chili pepper and Negro pepper was at its best on the 14th day whilst onion and ginger were stronger at week three. Onion, prekese and ginger gave the best microbial inhibition with Negro pepper and chili pepper inhibiting the least on the third week. Onion essential oil was more effective against *Staphylococcus aureus* followed by prekese, ginger and chili pepper with Negro pepper being the least effective. Products treated with essential oils of chili pepper, ginger, onion and the control were most acceptable by the taste panelists whilst Negro pepper and prekese essential oil treated products were least accepted on the grounds of taste and aroma. The use of chili pepper, ginger and onion effectively improved eating and keeping qualities of frankfurter - type sausages.

Key Words: Herbs/Spices, Frankfurter Sausages, Kumasi, Phytochemicals, Antioxidants, Antimicrobials

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CHAPTER ONE

1.0 INTRODUCTION

Healthy animals are regarded as having sterile muscles since very few or no living microbes are present in the muscles. However, micro organisms are introduced on the surfaces of muscles when animals are slaughtered and dressed or processed. Microbial contamination of meat is therefore a continual possibility from the time of slaughtering through to consumption (Okubanjo *et al*., 2003 and Odey *et al*., 2013).

Besides the colonization of meat by spoilage microbes from slaughtering processes, breakdown of body homeostasis within the muscle fibres may also results in meat deterioration due to rancidity. Rancidity in meat often begins with the phospholipids located in cell membranes, although there is virtually a small quantity (~ 1%) of phospholipids in meat lipids. Meat spoilage mainly occurs due to the inability of the carcass to combat intruding microbes.

In order to reduce the rate of microbial multiplication and meat deterioration, a hurdle technology approach is required. This includes hygienic slaughtering, clean handling with immediate processing and preservation (Berkel *et al.,* 2004). Some common preservative interventions include curing, smoking, salting, drying, vacuum packaging, canning, addition of plant extracts and chemical preservatives (Berkel *et al.,* 2004). Addition of oxidation inhibitors early in the formulation of the product is one of the surest ways of inhibiting oxidative rancidity in meat products. Chemical preservation is more popular with processed meat products since it offers a good microbial inhibition when combined with refrigeration (Cassens, 1994).

One challenge in the use of chemical (synthetic) antimicrobial preservatives is the promotion of resistant microbial strain to classic antimicrobial agents (Souza *et al*., 2005). Multi resistant strains difficult to control have been reported widely in literature (Souza *et al.*, 2005). Also, Levy (1997**),** reported on the ecological imbalance and enrichment of multiple resistant pathogenic microorganisms due to increased use of these chemical antimicrobial preservatives. In light of this, several spices and herbs as well as their extracts are being added in a variety of foods to improve their sensory characteristics and extend shelf-life (Shahidi *et al*., 1992**).**

However, most spices are known for their flavour enhancement rather than shelf life extension. Literature has several reports on the preservative properties, antioxidant, antimicrobial and medicinal potentials of spices and herbs. The components of essential oils in spices have been of great interest as they may be a rich source of antioxidants (Saowaluck and Paisooksantivatana, 2009), due to the presence of phenolic groups which enable them scavenge free radicals. Wang *et al.* (2008), reported on the antioxidant activity of essential oil obtained from *Rosemarinus officinalis* (rosemary).

Though spices are used in small quantities in meat product preparations, it is obvious that they are indispensable since they contribute to product flavour, product colouration, and product stability. There are few spices which have gained popularity in use despite the numerous plants species considered as spices. This probably may be due to the fact that several spices are uncommon to other cultures. There is therefore the need to promote both local and exotic spices in meat products manufacture.

Black pepper is an exclusively tropical plant that has several useful properties. It contains the compound piperine which inhibits the ubiquitous, deadly bacterium *Clostridium botulinum* (Nakatani, 1994). Black pepper is also a "bioavailability enhancer," i.e. acts synergistically to increase the rate at which cells, including microorganisms, absorb phytotoxins (Johri and Zutshi, 1992).

Several phytochemicals found in these spices, such as rosmarinic acid (Lee *et al.*, 2006) in thyme and oregano (Shan *et al.*, 2005), eugenol in clove and allspice (Chainy *et al.*, 2000) and gallic acid in clove, have all been identified as inhibitors of NF-κB, a transcription factor which is crucial in the orchestration of immune and inflammatory responses. An extract of clove, oregano, thyme, together with walnuts and coffee also inhibited NF-κB activation in a synergistic manner in vitro, and in vivo in transgenic mice (Paur *et al.*, 2010). Essential oil extracts are one of the naturally occurring compounds considered as natural preservatives or food additives that can be used in controlling pathogens (Naidu, 2000).

Of late, consumers are more informed and concerned about the processed food they consume, questioning the health implications of synthetic preservatives, which have been used in foods for years (Namiki, 1990). Also, the increasing preference for natural foods makes it obligatory for the food industry to replace synthetic antioxidants with natural antioxidants to delay oxidative degradation of lipids, improve quality and nutritional value of foods (Wojdylo *et al*., 2007 and Camo *et al.*, 2008).

The need for more research to determine antimicrobial activity and antioxidants properties of more natural spices (both local and exotic) in meat during storage and also to identify the main metabolic pathway of the compounds responsible for these effects on other meat quality parameters are of utmost importance (Velasco *et al.*, 2011).

The main objective of this study was to investigate the preservative (antioxidant and antimicrobial) effects of the most used spices/herbs on meat products within Kumasi Metropolis-Ghana. The specific objectives were to

1. Identify spices/herbs used in the Kumasi metropolis;
2. Assess the phytochemical properties of some of the spices/herbs and

3. Utilize the essential oils of these selected spices in frankfurter sausages for the control of lipid oxidation and microbial contaminations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Spices and Herbs used in the Meat Industry

Several plant parts are used as spices or herbs in the food and meat industries for various reasons. One major reason is to improve flavour of food items (Bauer *et al*., 2001). Over the years, it has been observed that spices and herbs could enhance the keeping quality, make food attractive, garnish and even prevent spoilage of food items.

According to Farrell (1990), a practical interpretation for the food industry that has gained wide acceptance and in essence has been adopted by the spice trade is that the term spices is applied to any dried, fragrant, aromatic or pungent vegetable or plant substance in the whole, broken or ground form that contribute flavour, whose primary function in food is seasoning rather than nutrition, and that may contribute relish or piquancy to food or beverage that is true to name and from which no portion of any volatile oil or other legal flavouring principle has been purposely removed or to which no additives or spent spice has been added. Redgrove (1933) also defined spices, as quoted by Davidson (2006), as dried parts of aromatic plants which include rhizomes, roots, barks, flowers, fruits and seeds. From the two definitions it is clear that the dried parts of aromatic plants used in food flavouring are spices. This definition is also supported by Savic (1985). As aromatic plant materials, they have been used in food preparation and preservation, as well as for embalming, in areas where the plants are native, such as Hindustan and the Spice Islands (Govindarajan, 1985 and Farrell, 1990).

Generally, a culinary herb typically refers to fresh or dried leafy part of a plant used in cooking. Common herbs include mint, chives, chervil, rosemary, thyme, dill, green basil, lovage, oregano and many others. Any other part of the plant, often dried, is considered as a spice. For instance, clove is a bud, cinnamon is a bark, ginger is a root (rhizome), peppercorns is a berry; cumin is a seed and saffron the stigma of a flower (Farrell, 1990; Tapsell *et al.,* 2006).

2.1.1 Spices/Herbs Commonly Used in Kumasi Metropolis

Kumasi is the second largest city of Ghana, located in the middle belt of the country. It covers a total area of 254 sqkm. It is a cosmopolitan city with estimated resident population of 1,915,179 and inter censal growth of 5.4% (MTDPKMA, 2010-2013). The multi- cultural representation of the people living in the city is reflective in the use of spices/herbs. Some of the most common spices/herbs found in the city include Chili Pepper, Garlic, Onion, Ginger, Aniseed, Nutmeg, Prekese, West African Black Pepper, African Locust Bean just to mention a few.

2.1.1.1 Ginger (Zingiber officinale)

Ginger (*Zingiber officinale*) is a member of the family of plants called *Zingiberaceae.* Cardamom and turmeric are other spices found in the same family. It is grown chiefly in Asia and tropical areas. Its underground root or rhizome has been used for culinary and medicinal purposes since ancient times for a variety of conditions, including colds, fevers, digestive problems, stomach upset, asthma, diabetes, menstrual irregularities and also as an appetite stimulant (Langner *et al*., 1998; Wang *et al*., 2005; Tapsell *et al*., 2006; Ali *et al*., 2008). Although, the U.S. Food and Drug Administration categorizes ginger as a food additive and studied for the treatment of nausea and vomiting, as well as arthritis (White, 2007).

Zhou and Xu, (1992) and Pearson *et al*. (1997), reported significant antioxidant effects of zingerone on low density lipoproteins in aortic endothelial cells of humans. Ginger extract, according to Sujatha and Srinivas, (1995), also inhibited lipid peroxidation in human erythrocyte membranes by 72%.

Ginger of African origin is darker in color and higher in monoterpene content. This gives it a more pungent aroma with camphoraceous notes. It has a high oil content and level of pungency; therefore it is usually preferred for the production of oils and oleoresin (Sutarno *et al*., 1999 and Weiss, 2002).

The rhizome can be processed into a powder, syrup, volatile oil, and oleoresin. The rhizome contains fats, carbohydrates, protein, fiber, water, and volatile oil. Cultivation practices and postharvest treatment of ginger affect the quality and quantity of its biologically active constituents. And depending on the location of cultivation and whether the product is fresh, dried, or processed, the chemical components can also vary considerably (Ali *et al*., 2008).

The pungency of fresh ginger results from a group of phenols, of which gingerol is the most abundant (Chubrasik *et al*., 2005). It may also contain a 5-deoxy derivative of ginger called paradol. Dry ginger, however, exhibits pungency due to shogaols, (dehydrated forms of gingerols) resulting from thermal processing (Connell and Sutherland., 1969). Other substances that may be contained in ginger include volatile oil (imparts a distinctive odour to ginger). This volatile oil in ginger is composed mainly of monoterpenoids and sesquiterpenoids, including camphene, borneol, zingiberene, sesquiphellandrene, and bisabolene (Langner *et al*., 1998; Chubrasik *et al*., 2005 and Ali *et al*., 2008). Eleazu *et al*. (2012), reported on the phytochemical composition of the leaves of ginger as follows; Saponin-0.80±0.000%, Flavonoid -

6.70±0.424%, Alkaloid- 6.00±0.283%, Tannin - 0.43± 0.004mg/100g, and Cyanogenic glucosides 0.62±0ug/g. Bhargava *et al*. (2012) also reported that crude extract of ginger roots contains abundant alkaloids, saponins; moderate amount of tannins, glycosides and flavonoids with fair amount of terpenoids and no steroids. Belewu *et al*. (2009), on the other hand reported the presence of tannins and saponins with the absence of alkaloids, polyphenols, flavonoids and sterols in ginger.

2.1.1.2 Onion (Allium cepa)

Onion belongs to the family *Alliaceae* and genus *Allium* along with a great number of other species of similar odour and taste, namely garlic, chives, shallots, leeks and welsh onion. Onions are grown chiefly as green onions or dry bulbs. Asia is considered the centre of origin in countries such as India, Afghanistan, USSR and China. The knowledge of onion and their cultivation began from the earliest period of history and believed to be one of first cultivated crops (Benkeblia, 2004).

Onions vary in shape, color and taste. Bulbs can be round, flattened, or torpedo- shaped, and white, yellow or red, sweet or pungent. It is widely consumed due to its flavouring and health-promoting properties. Block (1985) reported the potency of onion extract as cardiovascular and anticancer agent with hypocholesterolemic, thrombolitic and antioxidant effect. Several antioxidant compounds, mainly polyphenols such as flavonoids and sulphur-containing compounds have been identified in onion (Nuutila *et al*., 2003). More research has focused on one of the flavonoid called quercetin. Quercetin is found at high levels in onions and functions as an antioxidant to deactivate molecules that are injurious to cells in the body. Research studies have also shown that quercetin decreases cancer tumor initiation, promotes healing of stomach ulcers and inhibits the proliferation of cultured ovarian, breast, and colon cancer cells. The organosulfur compounds are largely responsible for the taste and smell of onions. Researchers have shown that organosulfur compounds reduce symptoms associated with diabetes mellitus inhibiting platelet aggregation (involved in thrombosis) and prevent inflammatory processes associated with asthma (NOA, 2015).

Whitemore and Naidu (2000) also reported the antibacterial and antifungal activities of onion against a variety of Gram-negative and Gram-positive microbes. Further investigations have also demonstrated an inhibitory effect by aqueous extracts on numerous bacterial and fungal species (Hsieh *et al*., 2001; Ward *et al*., 2002).

Abuga (2014) reported that onions contain the following secondary metabolites: saponins, flavonoids, tannins and cardiac glycosides but had negative result for steroids and alkaloids. Glycosides, alkaloids, saponins, flavonoids were present while no sterols were found in onion (Ugwoke and Ezugwe, 2010).

2.1.1.3 Chili Pepper (Capsicum frutescens)

Chili pepper is native to the Central American region but was introduced to other parts of the world mainly by the Spanish and the Portuguese. It is a perennial plant of the *Solanaceous* family belonging to the genus Capsicum. Chili pepper fruits synthesize and accumulate a variety of compounds, which include capsaicinoids (hot compounds), vitamins (Vitamins A, B and C), and pigments (anthocyanins and carotenoids) (GómezGarcía and Ochoa-Alejo, 2013). It contains alkaloid compounds like capsaicin which gives it a spicy pungent character. Capsaicin has antibacterial, anti-carcinogenic, analgesic and anti-diabetic properties. Capsaicin also reduces LDL (Low Density Lipoprotein) cholesterol levels in obese individuals and also serves a potent inhibitor of substance P - a neuropeptide associated with inflammatory processes.

The fruits have antioxidant effect which is affected by the ripening stage. Fully ripe chili pepper fruits exhibit higher antioxidant activity than green fruits due to the increased amounts of carotenoids, phenolics, flavonoids and ascorbic acid in the fully ripe fruits (Sun *et al*., 2007). Phytochemical screening conducted by EmmanuelIkpeme *et al*. (2014), showed the presence of tannins, flavonoids, alkaloids, phenolics, saponins, glycosides, terpenoids and other secondary metabolites in chili pepper.

2.1.1.4 Negro Pepper (Xylopia aethiopica)

Xylopia also known as Negro pepper, grains of Selim, kani pepper, moor pepper, West African pepper tree, and Senegal pepper is native to Africa, but largely found in West, Central and South (Ghana, Nigeria, Senegal, Democratic Republic of Congo, Ethiopia, Kenya, Mozambique, Tanzania, Uganda). Vernacular names for *Xylopia aethiopica* in the sub region are Hwentia-Ghana, Sesedu, Kimba-Nigeria, Fonde-Ivory Coast, Konde, Berbere -Ethiopia, , Hewe -Sierra Leone, Akatapure - Togo (Osazuwa *et al*., 2014). The tree which is an angiosperm of the *Annonacea*e family is widely spread in the humid forest zones especially along rivers in the drier area of the region (Orwa *et al*., 2009 and Woode *et al*., 2012).

The fruit of *Xylopia aethiopica* is found to contain kaurane derivatives which are effective for treating several medical conditions (Tairu *et al*., 1999 and Barminas *et al*., 1999). It is used as a cough remedy, post-partum tonic, anti-malarial and for the treatment of amenorrhea and uterine fibroid. Decoction of the seeds of Negro pepper is also used to induce placental discharge postpartum (Burkhill, 1985). The fruits have antimicrobial, and antifungal activity, possess haematopoietic activity, androgenic and spermatogenic activity (Boakye-Yiadom, 1977; Taiwo *et al*., 2009 and Woode *et al*., 2011).

Osabor *et al*. (2015), stated that the levels of phytochemicals present in *Xylopia aethiopica* are as follows: Alkaloids (2.40±0.1%), flavonoids (8.10±0.1%), saponins (1.36±0.01%), polyphenols (3.88±0.03%), reducing sugars (glucose and fructose) (44.50±0.1%). Upon using ethanol and water as a solvent media for phytochemical screening, the following result was obtained: alkaloids, glycosides, saponins, flavonoids, reducing sugars such as (Glucose and fructose), polyphenols, triterpenes and steroids (Osabor *et al*., 2015).

2.1.1.5 Aridan Plant (Tetrapleura tetraptera)

Tetrapleura tetraptera belongs to the family *Mimosaceae* and is generally found in the lowland forest of tropical Africa. The common name is Aridan with the following as vernacular names: prekese- Ghana, aidan- Nigeria (South - Western), Munyegenye - Uganda (Katende *et al*., 1995; Soladoye, *et al*., 2012).

The fruit possesses a fragrants, characteristically pungent aromatic odour and is conventionally used as spice and as a natural multivitamins. It is rich in protein, lipids, potassium, iron, magnesium, phosphorous, and vitamin C (Aladesanmi, 2007 and TFNet, 2011).

The therapeutic properties of *Tetrapleura tetraptera* has been documented far back in 1948 and authenticated in laboratory and field experiments. In Nigeria, it is cooked in soup and fed to mothers to prevent post-partum contraction. In Ghana, soft drinks flavoured with prekese have been approved by the Food and Drugs Board, and are marketed to reduce hypertension, decrease the severity of asthma attacks, and promote blood flow (TFNet, 2011). It is one of the molluscidal medicinal plants of Nigeria, useful in the management of convulsions (due to its ability to slow down the central nervous system), leprosy, inflammation and rheumatoid pains (Aladesanmi, 2007 and TFNet, 2011). The fruits are used to prepare food for mothers upon delivery to prevent postpartum contraction (Nwawu and Akah, 1986). Prekese extract has been reported to reduce the risk of certain types of ulcer and also inhibit the growth of bacteria.

Powdered dried fruits are added in soap bases to provide for anti-microbial properties (TFNet, 2011).The fruits of *Tetrapleura tetraptera* have strong radical scavenging and reducing capacities (Badu *et al*., 2012).

In other developments, _prekese' pod powder was used as a spice in sausage and hamburger production and yielded positive results in the sensory and nutritional qualities of the products (Lartey, 2012 and Adu-Adjei *et al*., 2014). Uyoh *et al*., (2013) reported that prekese contains tannin (0.24 to 0.64%), sterol (0.04 to 0.14%), phenol (0.05 to 0.12%), saponin (0.44 to 0.8%). alkaloid (1.73 to 2.76%) and flavonoid (1.63 to 3.84%).

2.2 Physicochemical Properties of Spices and Herbs

The versatile use of spices and herbs is dependent on their physical and chemical properties. Different spices/herbs contain various chemical constituents that enable them to offer piquancy and preservative properties to meat products. Literature has reported severally on some phytochemical properties of spices and herbs (Doughari *et al*., 2009).

2.2.1 Phytochemical Properties of Plants

The word *phyto*- originates from Greek and it means _plant'. Phytochemicals therefore refer to non-nutrient chemicals in plants that have protective or disease and pest preventive properties (Nweze *et al*., 2004 and Doughari *et al*., 2009). They are not essential nutrients because they are not required by the human body for life sustenance. Plants produce phytochemicals to protect themselves; however, research has demonstrated that they can also protect humans against diseases (Ani, 2008 and

Doughari *et al*., 2009). A lot of phytochemicals do exist. Among the well-known phytochemicals are flavanoids in fruits, lycopene in tomatoes and isoflavones in soyabean. Different phytochemicals work in different ways.

Some imitate human estrogens and help to reduce menopausal symptoms and osteoporosis, an example is isoflavones, found in soyabean. Some phytos are enzyme stimulants. For instance indoles, found in cabbages, stimulates enzymes that make estrogen less effective and could reduce the risk for breast cancer. Also, terpenes found in citrus fruits and cherries interfere with enzymes. Saponins found in beans interfere with the replication of cell DNA, and prevent multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens. The phytochemical - allicin from garlic has anti-bacterial properties (Heinrich *et al*., 2004).

2.2.1.1 Essential Oil Content in Spices and Herbs

Essential oils are natural aromatic compounds found in the seeds, bark, stems, roots, flowers, and other parts of plants and are generally obtained by steam or water distillation (NAHA, 2015). These oils contain the true essence of the plants from which they are derived. These oils are non water-based phytochemicals consisting of volatile aromatic compounds. They are fat-soluble but essential oils do not contain fatty acids/lipids contained in vegetable and animal oils. Pure, unadulterated essential oils are very clean, translucent and range in color from crystal clear to deep blue.

They are very much concentrated and a little usage gives effective response.

The chemical composition and aroma of essential oils can provide valuable psychological and physiological therapeutic benefits when applied on the skin diluted or inhaled.

In recent years, scientific research has shown that plants produce essential oils for a variety of purposes. While still in the plant, essential oils constantly change their chemical composition to help the plant adapt to the ever-changing internal and external environment. By this, they offer protection for plants against predators and diseases. They aid the attraction of pollinators and dispersal agents due to their distinctive smells. For instance various insects, including bees, butterflies, and even beetles, are known to be attracted by the scent or aroma of a plant (NAHA, 2015).

They also defend the plants against insects and other animals. Plants use terpenoid compounds to deter insects and other animals from approaching them. Shawe (1996) reported that peppermint plants and peel of citrus fruits resist Caribbean fruit fly attack due to the presence of linalool. Also, a complex mixture of volatile oils or terpenes produced in the thorns of the Douglas fir tree defends the tree against the spruce budworm. The tree can even vary the composition and production of terpenes each year to decreasing the ability of the budworm to develop widespread immunity to specific compounds (Buhner, 2002).

Essential oils offer protection for plants against fungi and bacteria. Complex combinations of terpenes and resins are released by some plants to act as antimicrobial agent against a wide range of microorganisms that threaten plant survival. Sesquiterpene lactones found in some plants (e.g.blessed thistle) provide a protection for plants against herbivores (NAHA, 2015).

Essential oil extracts of spices and herbs contain several chemical substances which enable them offer antimicrobial, antibacterial, antifungal and antioxidants activities to substances treated with these oils. Benkeblia (2003) reported that the essential oil extracts from Allium plants (garlic and onions) exhibited marked antibacterial activity.

Literature has it that the intense _pepperish note‘ of the oil of *Xylopia aethiopica* largely comes from the linalool it contains and it is this same chemical compound that provides the characteristic aroma of the ground, dried, smoked fruits of *Xylopia aethiopica*. The bark oil of this plant consists mainly of α- pinene, transpinocarveol, verbenone and myrtenol. The bark oil differs significantly from that of the leaf oil which contains spathulenol, cryptone, beta-caryophyllene and limonene. Wang *et al*. (2008) reported that the strong antioxidant activity of *Rosmarinus officinalis L.* essential oil is as a result of positive interactions between the components of the oil.

2.2.1.2 Bioactive Substances

2.2.1.2.1 *Saponins*

Saponin is a term derived from *Quillaja saponaria* - a plant, which abounds in saponins and was once used as soap. Saponins have _soaplike‘ behaviour in water, and thus, produce foam. There are two major groups of saponins. These are the steroid saponins and triterpene saponins. They are soluble in water but insoluble in ether. On hydrolysis, they produce aglycones, thus, similar toglycosides (Doughari, 2012). According to Kar (2007), saponins are extremely poisonous, causing heamolysis of blood and cattle poisoning. They are bitter and acrid in taste and cause irritation to mucous membranes. The general structure of saponin is as shown in figure 1 below.

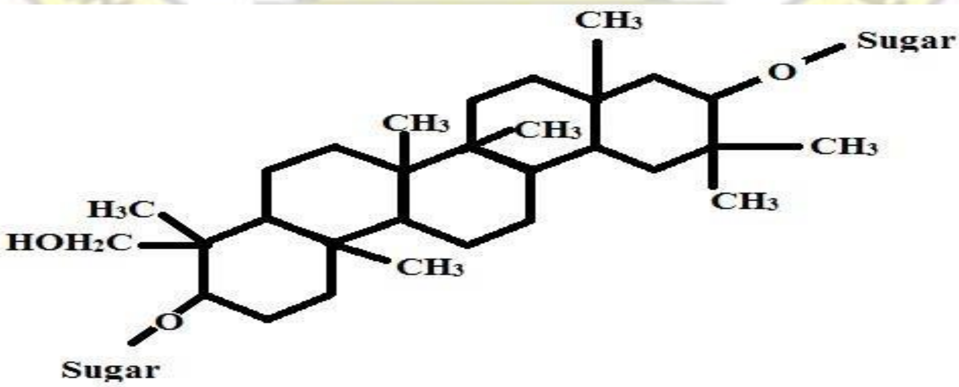


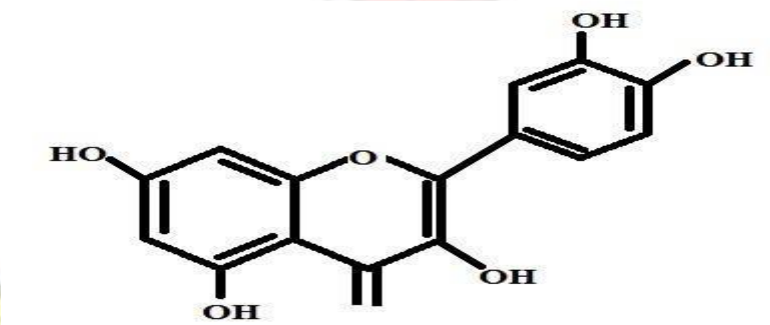
Fig.1: Structure of Saponin (Source: Liu et al., 2004)

2.2.1.2.2 Flavonoids

These are polyphenols commonly found in plants. They are aromatic compounds used

flavans, with over four thousand flavonoids known to exist. Some flavonoids are pigments in higher plants. According to Doughari (2012), about 70% of plants contain flavonoids namely, quercetin, kaempferol and quercitrin. The structures of some flavonids (quercetin and kaempferol) are shown in figure 2 below.

a) Quercetin



b) kaempferol

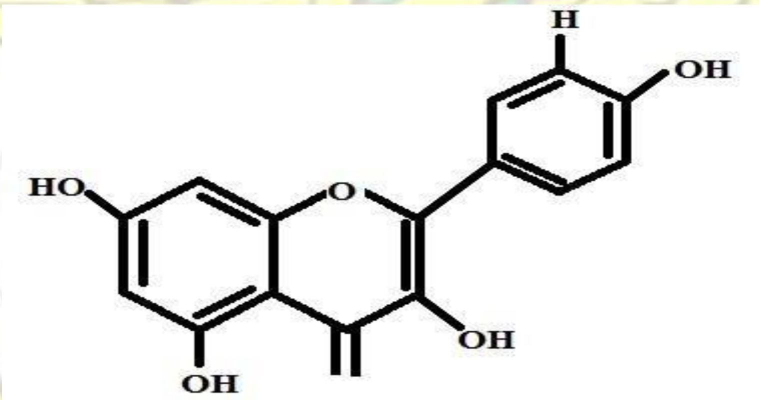


Fig.2: Structures of some flavonoids (Source: Jia and Lui, 2013)

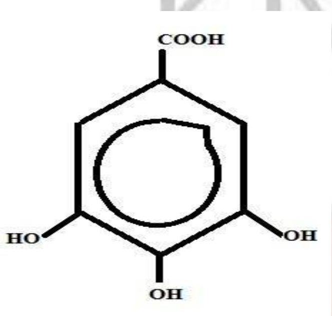
2.2.1.2.3 *Tannins*

These are widely distributed phenolic compounds of high molecular weight found in

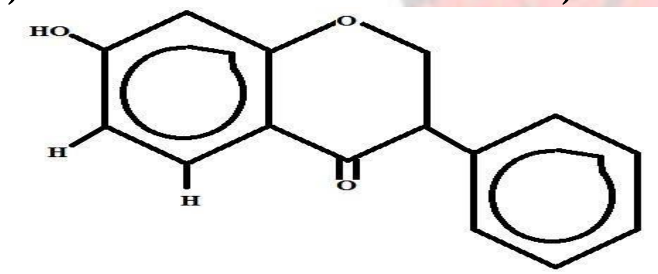
as antioxidants or free radical scavengers (Kar, 2007). Flavonoids are derivatives of the root, bark, stem and outer layers of plant tissue. Tannins are soluble in water and alcohol and have the ability to convert hide and skin of animals into leather (i.e. tan).

The presence of phenolics or carboxylic group in tannins makes them acidic in reaction (Kar, 2007) and used as antiseptic. Tannins may be classified as hydrolysable or condensed based on their structure. Hydrolysable tannins produce gallic acid and ellagic acid. Common examples of hydrolysable tannins include theaflavins, daidezein, genistein and glycitein. The structures of some hydrolysable tannins are shown in figure

3a.



i) **Gallic Acid** **ii) Daidzein**



iii) Glycitein

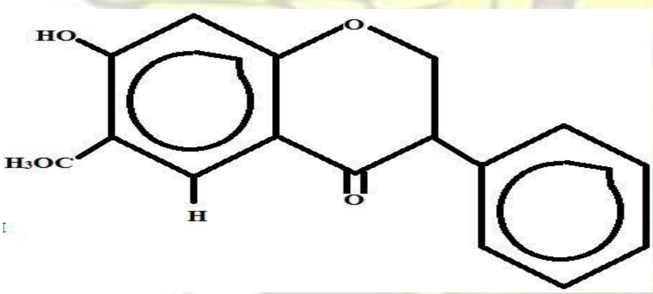
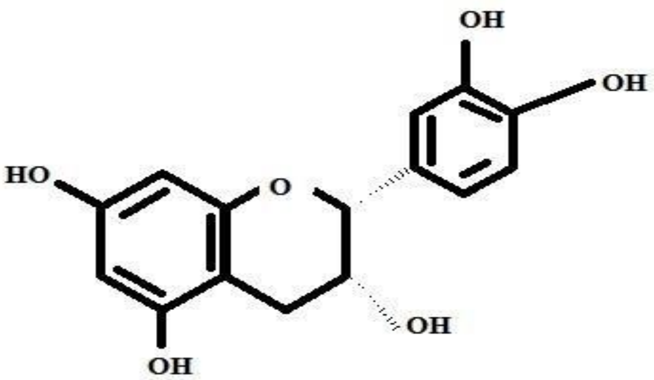
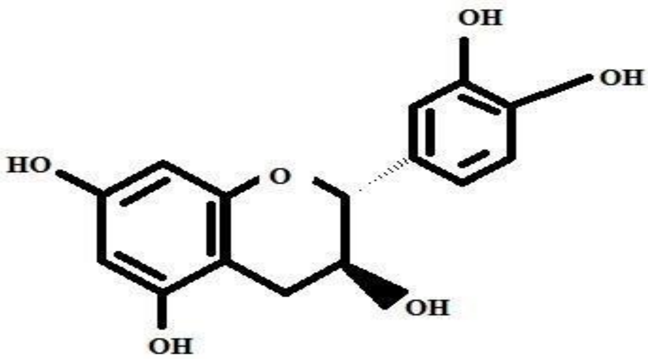


Fig.3a: Structures of Hydrolysable Tannins (Source: Doughari, 2012)

Condensed tannins, also called proanthocyanidins (PA), are polymeric flavonoids (Wünsch *et al*., 1984) which yield anthocyanidin pigment upon oxidation cleavage in hot alcohols like acid butanol (Hagerman, 2002). An impo rtant group of condensed tannins are 5 - deoxy-flavon-3-ols polymers. The most widely studied ones are the flavon-3-ols (-)-epicatechin, (+)-catechin and some low-molecular weight proanthocyanidins due to their various biological activities, such as their effects on arteriosclerosis (Masquellier, 1988) and their oxygen free radical scavenger ability (Ricardo-da-Silva *et al*., 1991). Plants rich in tannins are used as healing agents in a number of diseases like leucorrhoea, rhinnorhoea and diarrhoea. The chemical structures of some condensed tannins are shown in figure 3b. hence applied in the



i) Epicatechin **ii)**



Catechin

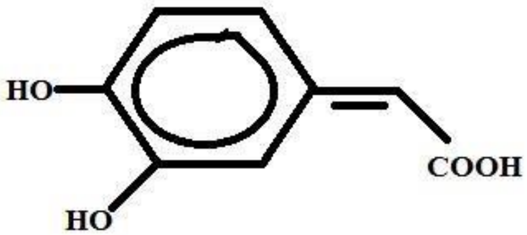
Fig.3b: Structures of Condensed Tannins (source: Natsume et al., 2003)

2.2.1.2.4 *Phenolics*

Phenolics are natural colour pigments for fruits of plants.Phenolics in plants are mostly synthesized from phenylalanine through the action of the enzyme phenylalanine ammonia lyase (PAL). They protect plant against pathogens and herbivore predators,

control of pathogenic infections in humans. Caffeic acid is a common example of phenolic compounds found in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, 2007).

Phenolics essentially represent a host of natural antioxidants that are used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer. They are also thought to prevent heart diseases to an appreciable degree and also as anti-inflammatory agents (Kar, 2007). The structures of phenolics such as caffeic acid and flavone are shown in figure 4 below.



a) **Caffeic acid** b) **Flavone**

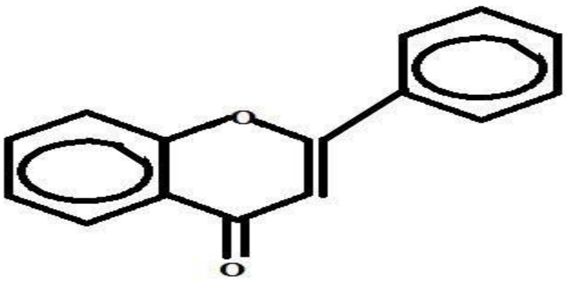


Fig. 4: Structures of Some Phenolics (Source: Doughari, 2012)

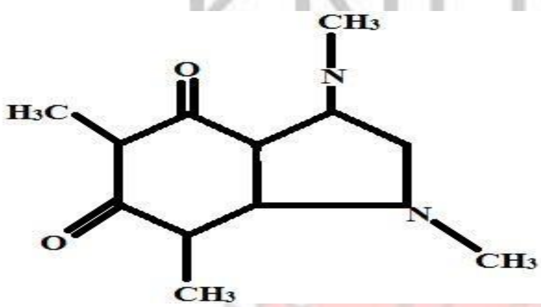
2.2.1.2.5 *Alkaloids*

Alkaloids are found in plants (seeds and roots) . They form secondary chemical constituents made of ammonia compounds. They have basic properties and turn red litmus paper blue. The basic properties of alkaloids are due to one or more nitrogen atoms present in the compound. According to Sarker and Nah ar (2007), the degree of basicity varies depending on the structure of the molecule as well as the presence and location of the functional groups. Upon reaction with acid, alkaloids form crystalline salts (Firn, 2010).

The solutions of alkaloids are intensely bitter and function in defense of plants against herbivores and pathogens and thus, act as antibacterial and antifungal and also against other plants by means of allelopathically active chemicals (Molyneux *et al*., 1996).

Owing to the potent biological activities of alkaloids, they are exploited widely as

Figure 5 below shows the chemical structures of some alkaloids.



a) Caffeine **b) Sanquinarine**

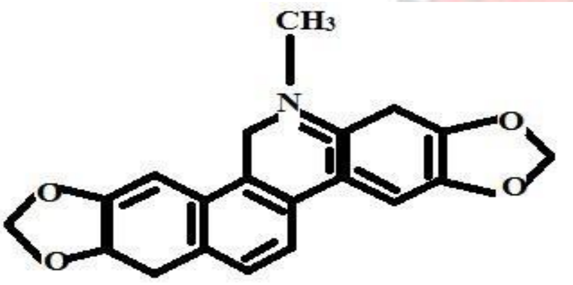


Fig.5: Structures of Some Alkaloids: (Source: Doughari, 2012)

2.2.2 Nutrient Composition of Some Spices and Herbs

Zadeh and Kor (2014), reported on the nutritional composition of fresh ginger as follows: moisture-80.9%, protein - 2.3%, fat- 0.9%, minerals- 1.2%, fibre - 2.4% and carbohydrates - 12.3%. The powdered rhizome contains 3.55 % -12% moisture, 5.02 - 9% protein, 0.76- 6% fatty oil, about 3.38 - 8% ash, 3-8% crude fiber, 60-70% carbohydrates, and 2-3% volatile oil (Sangwan *et al.*, 2014; Zadeh and Kor, 2014). The minerals in ginger include iron, calcium and phosphorous. Vitamins such as thiamine, pharmaceuticals, stimulants, narcotics, and poisons. They have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al*., 2010). Some important alkaloids of plant origin include caffeine, nicotine, ergotamine, cocaine and ephedrine. riboflavin, niacin and vitamin C are also present. It must be noted that the nutrient composition of ginger varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions.

Grubben and Denton (2004) and Bhattacharjee *et al*. (2013), reported that a fully developed onion bulb usually contains 82.77 % - 88.6% moisture, 1.2-2.0 % protein, 0.1-0.721% fat, 0.6-1.0% fibre, 0.4-0.7% mineral elements and 10.2- 14.772% carbohydrates. The mineral content includes potassium, phosphorus, sodium, calcium, iron copper, zinc and manganese. Onion is low in energy and provides vitamin C, folate, niacin, thiamine, riboflavin, vitamin B6, B1, and abundant flavour (Sullivan *et al*, 2001; Hedges and Lister, 2007).

Uyoh *et al*. (2013) reported the following as the proximate composition of *Tetrapleura tetraptera;* protein-5.48 to 7.84%, fat -11.79 to 21.71%, fibre - 2.79 to 4.81%, ash - 2.86 to 4.81% and carbohydrate - 51.17 to 66.29%. They also reported that the mineral consisted of zinc and potassium. "Prekese" contains vitamins A and C as well (Uyoh *et al*., 2013).

According to USDA, (2008), fresh red chili pepper contains 84.83g moisture, low energy of 24 calories, 0.91g protein, 5.55g carbohydrates, 1.8g fiber, and 0.28g fat.

Also chili pepper contains 6mg calcium, 0.40mg iron, 11mg magnesium, 24mg phosphorus, 194mg potassium, 2mg sodium, and 0.23mg. It also contains 174.8mg vitamin C, 0.050mg Thiamin, 0.078mg Riboflavin, 0.901mg niacin, 0.292mg pantothenic acid, 0.268mg vitamin B6, 17mcg folate, 2881mg vitamin A, 1.45mg Vitamin E and 4.5 mcg Vitamin K (USDA, 2008).

The nutritient composition of *Xylopia aethiopica*, were found to be as follows; moisture - 8.43g/100g, crude protein- 12.45g/100g, crude lipid- 9.58g/100g, crude fibre- 8.66g/100g and carbohydrate - 63.65g/100g, ash- 5.89g/100g (Barminas *et al*., 1999). The author reported also that calcium and potassium were the major minerals in the *Xylopia* seed. Abolaji *et al*. (2007) also reported on the nutritional composition of negro pepper stating the following values; Moisture 16.04±1.25%, protein 2.1±0.20%, fat 9.55±2.10%, Crude Fibre- 12.14±0.70%, ash- 4.37±0.85% and carbohydrate 55.80±4.26%. The minirals were magnesium- 2.236±0.095%, phosphorus - 0.620±0.04% and potassium- 0.510±0.04%. Calcium, zinc and sodium were other mineral elements detected in reasonable amounts. The results of this research indicated that the plant has nutritional qualities that could provide users with additional nutrients.

2.2.3 Functional Properties of Spices and Herbs

2.2.3.1 pH

The pH value of a food is a direct function of the free hydrogen ions present in that food. Acids present in foods release these hydrogen ions, which give acid foods their distinct sour flavor. Thus, pH may be defined as a measure of free acidity of a substance. More precisely, pH is defined as the negative log of the hydrogen ion concentration. The pH value of a particular food may have a dramatic effect on the type of processing needed to safely preserve it.

2.2.3.2 Swelling Properties

The swelling index is the volume in ml taken up by the swelling of 1g of herbal material under specified conditions (WHO, 2011). Swelling properties of many herbal materials are responsible for their utilization for specific therapeutic or pharmaceutical applications. Such properties may be as a result of the presence of gums, mucilage, pectin or hemicellulose.

The mixing of whole herbal material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent. The mean value of triplicate determinations is then calculated relating to 1g of the herbal material (WHO, 2011).

2.2.3.3 Oil and Water Absorption Capacities

Oil absorption capacity is useful in structure interaction in food especially in flavour retention, improvement of palatability and extension of shelf life particularly in bakery or meat products (Adebowal *et al*., 2005).

Water absorption is the amount of water taken up by the flour to achieve the desired or workable consistency or optimal end result of dough. Imbibition of water is an important functional trait in foods such as sausages, custards and dough. It is determined by the protein content of the flour, the amount of starch damaged during milling and the presence of non-starch carbohydrates (Finney *et al*., 1987; Simmonds, 1989). It is usually defined based on flour weight. For example, 60% water absorption would mean 60lbs of water is required for every 100lbs of flour. Generally, oil and water absorption capacities of products may be affected by temperature and is due to the amylose to amylopectin ratio of the products in question (WPC, 1996).

2.3 Storage of Spices and Herbs

All spices should be stored under dry conditions and possibly in sealed containers. They should not be exposed to direct sunlight and should only be ground or crushed on the day of manufacture (FAO, 1990). Spice mixtures should not be kept for long before use. This is true for individual spices also, once they have been ground, but the principle applies morestrongly to mixtures since the various components will stay viable for different length of time, with the result that some will become stale before others and the balance will be disappointing (Davidson, 2006).

2.4 Meat and Meat Products

Meat can be defined as ―the muscle tissue of slaughter animals‖ (FAO, 2007) or all parts of an animal that are intended for, or have been judged as safe and suitable for, human consumption‖ (CAC/RCP, 2005). Meat is composed of water, protein and amino acids, minerals, fats and fatty acids, vitamins and other bioactive components, and small quantities of carbohydrates. With respect to nutrition, meat is important due to its high quality protein, presence of all essential amino acids and its highly bio available minerals and vitamins (CAC/RCP, 2005).

As sources of high quality protein and amino acids, meat and meat products compensate for the shortcomings in staple foods. They supply easily absorbed iron and assist the absorption of iron from other foods as well as zinc. Meat also serves as a rich source of vitamin B complex. It can therefore be agreed that, meat consumption can alleviate common nutritional deficiencies in the developing countries (FAO, 1992). In order to show variety in sensory characteristics of meat product, meat is processed into different products without much of a change in the nutritional composition. According to FAO (2007), processed meat products can be categorized with respect to the technology applied as follows:

- **Fresh Processed Meats** (hamburgers, fried sausages, kebabs, chicken nuggets)
- **Cured Meat Pieces** (raw ham, cooked ham, bacon and reconstituted products)
- **Raw Cooked Products** (frankfurter, mortedella, lyoner, and meat loaf)
- **Precooked Products** (liver sausages, blood sausages, corned beef)
- **Raw/Dry-Fermented Sausages** (salami) and
- **Dried Meat** (strips or flat pieces like biltong, beef jerkey and meat floss).

One category of interest to this project is Raw-cooked meat products. The component of this product which includes muscle meat, fat and non-meat ingredients are processed raw by comminuting and mixing. The resulting viscous batter is portioned in casings as sausages or otherwise and heat treated (cooked). The heat treatment induces protein coagulation which results in a typical firm-elastic texture for rawcooked products. In addition to the typical texture, the desired palatability and a certain degree of bacterial stability is achieved (FAO, 2007). Notwithstanding the bacterial stability of this category of meat products, it is worth recognizing and adhering to all the hygienic principles during preparation in the raw state in addition to any other preservative methods that will enhance longer shelf- life at storage.

2.4.1 Standards of a Wholesome Meat Product

All persons in contact with the food we eat have the responsibility to ensuring its safety from the farm to the dining table. This is so necessary for the fact that, no matter how effective one segment of the food industry may be, the effort of one cannot be compromised by the next segment in the food chain (Hale, 2002). According to Harris (2007), the meat industry is recognized as the most highly regulated of all food industries in the United States of America. At least there are about nine federal agencies which serve as ―watchdogs‖ to assure meat presented to consumers is wholesome and safe (Hale, 2002).

Since all the meat and meat products prepared are not consumed at a go, wholesome and safe meat products must be preserved. Preservation is based on slowing down or preventing spoilage by micro-organisms that are introduced along the processing line. According to Berkel *et al*. (2004), the dangers of micro-organisms can be avoided in three ways such as removal, killing or suppressing the activities of micro-organisms. The method of removing micro-organisms is very expensive to undertake and can only be used with liquids. On the other hand, micro-organisms are killed by the use of heat otherwise referred to as sterilization, whereby product can be stored for a long time, if the right temperature is kept. Meat products can also be pasteurized, whereby short heat (80℃) is applied to kill some of the microbes. Such products last for a limited period of time in storage. Moreover, microbes are suppressed when the environment in which they can no longer grow, or can grow only very slowly, is created. The various ways by which this can be done include, lowering the temperature, reducing the water content, increasing the osmotic pressure, and addition of preservatives to the products.

Keeping products at lower temperatures make meat remain fresh in the refrigerator (2-4℃) for 4-7 days. To store for much longer periods requires deep-freezing (-20℃). Low temperatures must be maintained accurately and continuously to ensure supply of quality food. Reducing the water content may result from drying which is the oldest way of preserving foods. When sufficient water is removed from a product, microorganisms can no longer grow. The amount of water to be removed varies with the product. The simplest and cheapest method is to dry the product in the open air (with or without sun). Somewhat more expensive and difficult methods make use of driers in which the products are artificially dried using heated air. Sun-dried products are of slightly less quality due to the break- down of certain vitamins in sunlight. Lengthy smoking is also based on the principle of reducing the internal water content and gives an added taste to the product.

Suppressing the activities of micro-organisms by increasing the osmotic pressure is a technique, in which salt is added at a higher rate to stop the growth of microorganisms. For instance meat and fish are salted. These preserved products keep well with reasonable nutritional value. Addition of certain substances can partly prevent spoilage. In practice, this method is only used as an aid for other preservation methods (Berkel *et al*., 2004).

2.5 Deterioration of Meat Products

Meat spoilage occurs due to a wide range of physical and chemical reactions as well as microbial or enzymatic actions on meat products. According to Berkel *et al*. (2004) and Dave and Ghaly (2011), the three basic mechanisms responsible for spoilage of meat are microbial growth, oxidation and enzymatic autolysis. Microbial growth on meat products depends on various factors such as pre-slaughter husbandry practices, age of the animal at the time of slaughtering, handling during slaughtering, evisceration and processing, temperature controls during slaughtering, processing and distribution, preservation methods, type of packaging and handling and storage by consumer. They also reported that, autoxidation of lipids and the production of free radicals are natural processes which occur enzymatically or non-enzymatically to affect fatty acids and result in oxidative deterioration of meat and off-flavour development.

2.5.1 Deterioration Caused by Lipid Oxidation

When spoiled food are consumed, they can cause symptoms such as diarrhoea, stomach pains, nausea, vomiting, stomach infections or cramps and even death in serious cases (Berkel *et al*., 2004). Lipid oxidation and the changes associated with it are the major causes of muscle food quality deterioration. This oxidation reaction is a complex process whereby unsaturated fatty acids reacts with molecular oxygen via a free radical chain mechanism and form fatty acyl hydroperoxides, also called peroxides (Gray, 1978). Several factors such as fatty acid composition, level of the antioxidant vitamin E (α-tocopherol) and pro-oxidants (free iron presence) in muscles contribute to oxidation of lipids in meat. Polysaturated fatty acids are more susceptible to lipid oxidation. Enser (2001) and Simitzis and Deligeorgis (2010), reported that lipid oxidation of highly unsaturated fatty acid fractions of membrane phospholipids produce hydroperoxides (susceptible to further oxidation). The reaction process involves primary autoxidation followed by a series of secondary reactions which lead to the degradation of the lipid and the development of oxidative rancidity. Lipid oxidation have gained much interest due to flavour deterioration, loss of nutritional value, ageing, functional property changes and the environmental pollution associated with it (Frankel, 1984).

Factors such as processing and storage conditions, type of ingredients and concentration of antioxidants, are very important in determining the rate of development and the possible deteriorative effects of lipid oxidation. Cooked meats stored in a refrigerator at 4°C can develop apparent rancid odours and flavours usually within 48h. These are particularly noticed after reheating the meat and are referred to as Warmed-Over Flavour (WOF) (Tims and Watts, 1958). Oxidised flavour in refrigerated cooked meats develops more rapidly compared to the slow onset of rancidity in raw meats, fatty tissues, rendered fat or lard, which is normally not apparent until they have been stored for weeks or months (Pearson *et al*., 1977). Temperature, water activity and pH also contribute to off flavour while non-enzymic browning reactions (Maillard) give rise to loss in nutritional value and organoleptic changes. Protein solubility, emulsification and water-binding capacity associated with texture and rheological properties, also appear to be affected by the interaction between lipid oxidation products and proteins (Hall, 1987).

Lipid oxidation is promoted by metals such as iron, cobalt and copper. These metals facilitate the transfer of electrons leading to increased rates of free radical formation (Ingold, 1962). The metal ions enter food mostly via the water used and sometimes via salt and spices (Taylor, 1987). Yong and Karel (1978), observed that inorganic iron and copper are strong catalysts of mackerel meat lipid oxidation; whereas Khayat and Schwall (1983), found that haem iron is the major catalyst of lipid oxidation in mullet fish. Verma *et al*. (1985) and Ledward (1987), revealed that, in raw meat and model emulsions, ferric haematin pigments are powerful catalysts of lipid oxidation.

Sato and Hegarty (1971), confirmed that any processes causing disruption of the muscle membrane system, such as grinding, cooking and deboning, results in exposure of the labile lipid components to oxygen, and thus accelerate development of oxidative rancidity. Destruction of the extremely well organized structure of living animal cells will bring together lipids, oxidation catalysts and enzymes responsible for lipid oxidation (Hall, 1987).

The control of oxidation has become increasingly important with increased consumption of prepackaged raw meat and precooked convenience meat items. According to Labuza (1971), a great variety of substances can exert antioxidant activities in meat products. These substances could be classified as free radical terminators whereby the substances donate hydrogen to the free radicals and stop chain reactions. Davidson (1993) and Simitzis and Deligeorgis (2010), listed phenolic compounds like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertbutyl hydroquinone (TBHQ) and tocopherol as examples of free radical terminators. Others are described as free radical preventors in the sense that these substances control the production of free radicals during ionization. Examples include metal complexing agents like ethylenediamine tetraacetic acid (EDTA), citric acid and phosphates. On the other hand, redox compounds (cysteine and ascorbic acid), water activity regulators, physical conditions and packaging material are described as environmental factors.

The extent of lipid oxidation in muscle foods may be checked following a variety of methods to measure either primary or secondary changes. The suitability of any method depends on the type of product and the way it has been processed and stored (Coxon, 1987). The selected method may also depend on the degree of correlation of the method with sensory analysis to be conducted (Igene *et al*., 1979).

2.5.1.1 The Use of Natural Nntioxidants to Stabilize Oxidation

The possibility of health risks from the use of additives in foods has gained much concern recently. Korczak *et al*., (1988) and Wang *et al*, (2008) reported in their experiments, where the oxidative stability of pork meat-balls in refrigerated storage was studied, that, rosemary and sage have strong antioxidant activity. The antioxidant activity of pepper investigated and confirmed by Milbourne (1987) showed that although it was the ethanol-soluble fraction that was exhibiting the antioxidant properties, piperine (the main component) does not seem to be the only ingredient of the fraction that could exert antioxidant properties. However, it was also stated that black pepper proved to be efficient inhibitor of oxidation.

According to Jurdi-EIaldeman *et al*. (1987), onion juice (20%) was more effective than garlic juice (4.8%) in reducing rancidity development in lamb, in both refrigerated and frozen cooked samples. Antioxidant activity of 10 spices (allspice, black pepper, cardamon, cinnamon, clove, coriander, cumin, ginger, nutmeg and rose petals) commonly used in the formulation of a fermented meat sausage were evaluated for their anti oxidative properties and clove, followed by rose petals and allspice, were found to exhibit the highest antioxidant index when used in a dry form (A1-Jalay *et al*., 1987).

A good number of methods have been used in estimating the level of lipid oxidation in meat products. One of such methods is the use of ***a, a***-diphenyl -***b***-picrylhydrazyl (DPPH), where ***a*** and ***b*** have numerical values of **1** or **2** as in 1, 1-diphenyl2picrylhydrazyl or 2, 2- diphenyl-1-picrylhydrazyl. DPPH is a stable free-radical darkcolored crystalline molecular powder whose major application is in laboratory research (Garcia and Luiza, 2012). The radical nature of a substance with antioxidant property can be tested by reacting it with DPPH solution. Researchers have pointed out that the molecule ***a, a***-diphenyl -***b***-picrylhydrazyl contains a decentralized electron which has the ability to increase violet colour as evidence of the antioxidant activity (Al - Termini and Choudhary, 2013). DPPH assays evaluate the ability of antioxidants to scavenge free radicals. One primary characteristic of antioxidants is the ability to donate a hydrogen atom (Andre *et al*., 2010). These hydrogen atoms donated convert the free radicals into non-toxic species and thus inhibit the propagation phase of lipid oxidation (Ordonez *et al*., 2006). When DPPH solution is mixed with any type of antioxidant that has the ability to grant a hydrogen atom, this mixture will have the ability to reduce violet colour as a sign of resistance to oxidative stress efficiently (Ruch *et al*., 1989). This change in colour is quantified by spectrophotometry absorbance reading (Yokozowa *et al*., 1998). The percentage of inhibition is calculated using the following equation: **Percent inhibition = [1-(As/Ao)] x 100,** where ***Ao*** is the absorbance of control and ***As*** is the absorbance of test (sample).

2.5.2 Deterioration Caused by Microbial Attack

The main sources of microorganisms in meat are the skin and the gut of the animals. The population and composition of microflora in meat depend on factors such as pre-slaughter husbandry practices (free range vs intensive rearing), handling during slaughtering, evisceration and processing, temperature changes, processing and distribution, preservation methods, type of packaging, handling and storage by consumer (Cerveny *et al*., 2009). Although the subsurface portions of meat are generally sterile, some parts such as lymph nodes may be heavily contaminated with microbes. These microorganisms could be distributed throughout the meat product as a result of mechanical disruption of the tissues during processing. Meat and meat products therefore provide an excellent growth media for a variety of microflora (bacteria, yeasts and molds) some of which are pathogens (Jay *et al*., 2005).

The major genera of microflora (bacteria, yeasts and molds) found in meat products before spoilage include

- Bacteria species - *Pseudomonas, Micrococcus, Streptococcus, Sarcina, Lactobacillus, Salmonella, Escherichia, Clostridium and Bacillus* (Lin *et al*., 2004 and Arnaut-Rollier *et al*., 1999).
- Yeasts species - *Candida spp., Cryptococcus spp. and Rhodotorula spp* (Garcia-Lopez *et al*., 1998)

- Mold species - *Cladosporium, Sporotrichum, Geotrichum, Penicillium and Mucor.*

Hayes *et al*. (2003), found *Enterococcus spp*. to be the most dominant bacteria on 971 of the 981 samples (99%) of all meat (chicken, turkey, pork and beef) in the state of Iowa. According to Cerveny *et al*. (2009), storage conditions affect the type of microbes found in meat and meat products. For instance *Enterobacteriaceae* are often present on refrigerated meat products while psychrotrophic lactic acid bacteria, *Enterococci, Micrococci* and yeasts are predominately found in raw, salted-cured products such as corned beef, uncooked hams and bacon due to their resistance to curing salts. GarciaLopez *et al*. (1998) also reported that the growth of *Enterobacteriaceae* and *Pseudomonas* were more prevalent on modified atmosphere packed meat (especially on pork) than on vacuum packed meat, their growth being favoured by storage at 5°C. Sentence (1991), shared similar view and reported that, *Pseudomonas spp.* growth rate was considerably slow at 0°C, but increased at 2°C and affected the shelf life of meat.

Sentence (1991), also noticed slow *Salmonella* growth below 7°C, which increased above 7°C and affected the shelf life of meat. The growth of lactic acid bacteria on bologna-type sausage was retarded 2 and 4 fold with decreases in temperature from 72°C and from 7-0.6°C, respectively (Borch *et al*., 1996). Russell *et al*. (1996), also observed that, the favorable pH for the growth of spoilage bacteria of meat ranges from 5.5 to 7.0. Slime formation, structural components degradation, off odours and appearance change are found in meat as a result of microbial growth loads exceeding 10^7 CFU/cm^2 within this pH range (Russell *et al*., 1996). Microbial consumption of meat nutrients, such as sugars and free amino acids and the release of undesired volatile metabolites results in the development of organoleptic spoilage. In chill-stored meat these activities may be performed at low temperatures by psychrotrophic bacteria, compromising the sole effect of temperature as affecting preservation (Ercolinia *et al*., 2009).

To determine the number of microorganisms in food products, a technique called Aerobic Plate Count (APC) is used (Cousin *et al*., 1992). Data obtained are often used as indicators of food hygienic quality or predictors for the shelf life of a product. The colonies of microbes formed are counted after inoculation and incubation of the sample aliquots, which only represent the organisms that could multiply at the given conditions of growth. The number of colonies that develop after an incubation period gives an indication of total microbial numbers. *Staphylococcus aureus, Salmonella, Escherichia coli* and *Pseudomonas aruginosa* are among the most deadly microorganisms that cause food and meat deterioration and also have pathogenic effect on the consumers.

2.5.2.1 Staphylococcus aureus

Staphylococcus aureus is Gram-positive and spherical in shape. The approximately 1 μm in diameter size bacterium has cells which form grape-like clusters due to its multiplane cell division. It is a facultative anaerobe capable of generating energy by aerobic respiration, and by fermentation to yield mainly lactic acid.

It is commonly found in commensal association with the skin and mucosae of humans and animals (Jay, 1997; Crossley and Archer, 1997) and is considered the third most important cause of diseases in the world amongst the reported food-borne illnesses (Zhang *et al.*, 1998). They are also found in the environment (Jay, 1997) as well as in some processing plants, such as poultry processing lines (Mead *et al*., 1989). As a result food products may become contaminated during or after processing leading to its isolation from several foods such as meat and meat products, chicken, milk and dairy products, fermented food items, vegetables, fish products, etc. (Tamarapu *et al*., 2001).

S. aureus is not only commensal but also a pathogen whose colonization significantly increases the risk of infections. This happens because the colonization provides a reservoir of the pathogen from which bacteria are introduced when host defense is compromised (Kluytmans *et al*., 1997). *S. aureus* is one of the main causes of hospital and community-acquired infections which can result in serious consequences (Diekema *et al*., 2001). Nosocomial *S. aureus* infections affect the bloodstream, skin, soft tissues and lower respiratory tracts. It also causes central venous catheterassociated bacteremia and ventilator-assisted pneumonia as well as serious deepseated infections, such as endocarditis and osteomyelitis (Schito, 2006). It is often responsible for toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome and staphylococcal foodborne diseases (SFD).

2.5.2.2 Salmonella typhi

It is a gram - negative, rod-shaped bacterium which causes food poisoning in human which results in gastroenteritis. The presence of *S. typhi* and paratyphi in meat products indicates human contamination and therefore poor personal hygiene during handling of the meat products (Mrema *et al*., 2006). The pathogen is associated with ill-prepared food such as uncooked beef, pork and lamb. Human infections with *Salmonella* are most commonly caused by ingestion of faecally contaminated food, water, or milk. Typhoid fever, caused by *S. typhi*, is a serious bloodstream infection common in developing countries. Bismuth Sulfite Agar is a highly selective and differential medium which is recommended for the isolation of *Salmonella* species, especially *S. typhi*, from food and clinical specimens.

2.5.2.3 Pseudomonas aeruginosa

Pseudomonas spp. are gram - negative rod bacteria highly adaptable to colonize various environmental niches, including soil and marine habitats, plants and animals.

Pseudomonas spp. are also opportunistic human pathogens which infect the eyes, ears, skin, urethra and respiratory tract in cystic fibrosis (CF) in burned patients and other immunocompromised individuals (Tashiro *et al*., 2012).

Pseudomonas spp. have also been reported to be involved in keratitis and fatal pneumonia (Huhulescu *et al*., 2011), among other diseases. Their presence may have a great impact on immune-suppressed individuals, since around 96% of the *Pseudomonas spp.* isolated from hot tubs and indoor swimming pools in a surveillance study display antimicrobial resistance (Lutz and Lee, 2011). Therefore, their prevalence in the environment, not only in recreational water but as part of biofilms in systems of distribution of drinking water, as well as their relevance in human pathogenicity led researchers to seek for its occurrence in amoeba hosts (Calvo *et al*., 2013).

2.5.2.4 Escherichia coli

Escherichia coli O157:H7 is a member of the enterohaemorrhagic E. coli (EHEC) group. It is a gram-negative, facultative anaerobic, non-spore-forming rod bacterium which was first identified as a human pathogen in 1982 when strains of a previously uncommon serotype, O157:H7, were implicated in two outbreaks of haemorrhagic colitis (HC) in the United States of America (Riley *et al*., 1983). *E. coli* is contracted by coming into contact (water or food) with the faeces, or stool, of humans or animals containing the bacterium. *E. coli* can get into meat during processing. If the infected meat is not cooked to 160°F (71°C), the bacteria can survive and infect the consumer upon eating the meat. This is the most common way people in the United States become infected with *E. coli*. Any food that has been in contact with raw meat can also become infected.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Organisation of the Project

The project was organized in two studies as follows:

1) a) Assessment of spice and herbs used in Kumasi metropolis - by administering a questionnaire (Study 1 Phase 1).

   b) Determination of phytochemical and functional properties of five most commonly used spices (two local and three worldwide) based on frequency of use (Study 1 Phase 2).

2) Formulation and evaluation of Frankfurter - type sausages treated with essential oils extracted from the selected spices/herbs (Study 2)

Study 1 Phase 1

3.2 Assessment of Spice/Herb Use in Kumasi Metropolis

A total of 400 questionnaires were administered to two groups of people namely:

1. Meat processors/vendors and
2. Consumers/domestic users of meat, spices and herbs.

Meat processors refer to people who process meat into products like sausages, burgers, meatloaves, kebabs and barbecues among others. Meat product vendors are those who sell meat products like *suya*, kebab etc. Meat product vendors usually add some form of preparation before the final sale of the product. Consumers and Domestic users refer to the final users of spices/herbs or the meat products. In all, 200 Processors/Vendors and 200 Consumers/Domestic users were purposively selected within the 10 sub metros of the Kumasi metropolis.

3.2.1 Data Collection: Two sets of structured questionnaires with two sections each were administered. One set for meat processors/vendors and the other set for domestic users/consumers of meat. Each set comprised of

- Demographic section (Section A) which required the age, religion, tribe, marital status and so forth of the individuals
- Section B: Required knowledge and use of spices by individuals as processors, vendors, domestic users or consumers.

3.2.2 Data Analysis**:** Data collected were subjected to descriptive analysis using SPSS (version 16.0 for Windows (2007). Results in pecentage forms were presented in tables.

Study 1 Phase 2

3.3 Phytochemical Screening of Selected Spices/Herbs

3.3.1 Experimental Site

Laboratory analysis was carried out at the Department of Pharmacognosy, and

Department of Food Science, all of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

3.3.2 Types and Sources of Selected Spices/Herbs **:** Five (5) most frequently used and most available spices/herbs used in the metropolis were bought from the Central Market- Kumasi. These materials were ginger, onion, chilli pepper, aridan plant (prekese) and Negro pepper as shown in Plate 1. All were obtained in the dried form except ginger and onion whose moisture contents were quite high. Ginger is a proven antioxidant and anti microbial agent in meat andother food items. It was therefore used as reference spice (ie positive control) in this study.

a) Onion

b) Ginge

c) Negro pepper

d) Prekese

e) Chili pepper

Plate 1: Sample of spices used in the study

3.3.3 Sample Preparation

The spices/herbs were treated as follows:

3.3.3.1 Sorting and Cleaning - Sorting was done to get rid of spoiled ones and other debris. Spices such as onion had it scaly leaves removed. They were then washed and cut into smaller pieces to facilitate faster drying. Ginger was however, washed of dirt and cut into smaller pieces with the scaly leaves on. This was done to prevent loss of fibre and essential oil content. Ginger and onion were sun dried until they were crispy.

3.3.3.2 Grinding - The samples were ground separately with thorough cleaning of the grinding device after each spice. The grinding was done using an electric coffee grinder (Philips Cucina-HR1751) and sieved through a micro-sieve of diameter 1mm.

3.3.4 Essential Oil Extraction

The essential oils of the spices were extracted from their ground samples by steam distillation using Clevenger apparatus for 5-6 hours as described by Satish (2010). Oils obtained were dehydrated with anhydrous sodium sulphate, placed into bottles rapped with aluminum foil and stored under refrigeration until used.

3.3.5 Qualitative Determination of Bioactive Factors

Bioactive factors such as phenolics, tannins, flavonoids, saponins, and alkaloids of the selected spices were qualitatively determined following procedures described by Mir *et al*. (2013) and Abuga (2014) as follows;

3.3.5.1 Phenols: Half (0.5) ml of Fe_2Cl_3 was added to 2ml of sample in test tube. Formation of intense green colour indicated the presence of phenols.

3.3.5.2 Tannins - Half (0.5) g of powdered sample of each spice/herb was boiled in 20ml of distilled water in a test tube. Filtered 0.1% $FeCl_3$ was added to the sample and observed for brownish green or a blue black colouration which showed the presence of tannins.

3.3.5.3 Flavonoids- A few drop of 1%NH_3 solution was added to aqueous extract of each sample in a test tube. A yellow colouration was observed indicating the presence of flavonoid.

3.3.5.4 Saponins- Two (2) g of powdered sample of each of spice/herb was boiled together with 20ml of distilled water in a water bath and filtered. 10ml of filtered sample was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain persistence froth. The frothing was then mixed with 3 drops of olive oil for the formation of emulsion which indicated the presence of saponins.

3.3.5.5 Alkaloids **-** Half **(**0.5) g of the each sample was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff‘s reagent were used to treat 1ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids.

3.3.6 Quantitative Determination of Phytochemicals

The quantitative analysis of the phytochemicals was done following procedures described by Boham and Kocipai-Abyazan (1994), Harbone (1973), Van-Burden and Robinson (1981) and Obadoni and Ochuku (2001) as follows:

3.3.6.1 Phenols - A fat free sample was obtained by defatting 2g of the sample with 100 ml of diethyl ether using a Soxhlet apparatus for 2h. The fat free sample was boiled with 50ml of ether to extract the phenolic component for 15min. Five (5) ml of the extract was pipetted into a 50ml flask, and 10ml of distilled water added. Two (2) ml ammonium hydroxide solution and 5ml concentrated amyl alcohol were also added. Samples were made up to mark and left to stay for colour development for 30min. Quantity of phenol was measured using spectrophotometer at 505nm.

3.3.6.2 Flavonoids - A solution was made from 10g of the ground sample using 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. The weight of the flavonoids was obtained and expressed as a percentage of the sample weight. Thus,

$$\%\ \text{flavonoid} = \frac{100(w2-w1)}{weight\ of\ sample},$$

Where **w1**- weight of empty crucible and **w2**- weight of crucible + flavonoids precipitate

3.3.6.3 Alkaloids- Five (5) g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue which is the alkaloid was dried and weighed.

3.3.6.4 Tannins - Five hundred (500) mg of the ground sample was weighed into a 100ml plastic bottle. 50ml distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50ml volumetric flask. Five (5) ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M$FeCl_3$ in 0.1M HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10min with a reagent blank at zero. Thus,

$$\% \text{ tannins} = \frac{100\ x\ AU\ x\ VF\ x\ D}{W\ x\ AS\ x\ VA},$$

Where, **W**= weight of sample used, **AU** = absorbance of standard tannin solution **AS** = concentration (mg/ml) of standard tannin solution, **VF** = total volume of filtrate **VA** = Volume of extract used, and **D** = Dilution factor necessary.

3.3.6.5 Saponins **-** Twenty **(**20) g of sample was dispersed in 200ml of 20% ethanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55℃.The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90 ℃. The concentrate was transferred into a 250ml separating funnel and 20ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether was discarded. The purification process was repeated. Sixty (60) ml normal butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage.

3.4 Functional Property Assays

Functional property assays of each selected spice/herb was determined as described by Adeleke and Odedeji (2010); Ikegwu *et al*. (2010) and Coffman and Garcia (1977) in triplicate as follows;

3.4.1 Residual Moisture Determination

The moisture content as left in the samples after sun drying to crispy was determined. Two (2) g of the ground sample was weighed into a previously weighed crucible. The

℃

crucible plus sample taken was then transferred into an oven set at 100 to dry to a constant weight. At the end of the drying, the crucible plus sample was removed from the oven and transferred to desiccators, cooled for 10min and weighed.

3.4.2 pH

Ten (10) g of ground form of each spice sample was homogenized in 50ml of distilled water in centrifuge tubes. The resulting suspensions were decanted and their pH determined using pH meter (model: Suntex pH meter, SP - 701) already standardized with buffer solutions of 4.0 and 7.0.

3.4.3 Water Absorption Capacity

Fifteen (15) ml of distilled water was added to 1g of the sample in a weighed 25ml centrifuge tube. The tube was agitated for 2min and centrifuged at 4000rpm for 20min. The clear supernatant was decanted and discarded. The adhering drops of water removed and then reweighed. Water absorption capacity of each sample was expressed as the weight of water bound by 100g dried sample.

3.4.4 Oil Absorption Capacity

Ten (10) ml refined corn oil was added to 1g of the flour in a weighed 25ml or centrifuge tube. The tube was agitated for 2min and centrifuged at 4000rpm for 20 min. The volume of free oil was recorded and decanted. Oil absorption capacity was expressed as volume of oil bound by 100g dried sample.

3.4.5 Swelling Power

The method of Okaka and Potter, (1977) with some modifications was used for determining the swelling capacity. A graduated cylinder was filled with the sample up to 10ml and water added to adjust the total volume to 50ml. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension was inverted again after 2min and allowed to stand for 30min more. The volume occupied by the sample was taken after 30 min.

Study 2

3.5 Formulation and Evaluation of Frankfurter type Sausages

3.5.1 Experimental Site

Frankfurter sausages treated with the essential oils from Negro pepper- *Xylopia eathiopica,* chili pepper - *Capsicum frutescens,* prekese - *Tetrapluera tretraptera,* onion - *Allium cepa and* ginger - *Zingiber officinale* were manufactured from pork at the Department of Animal Science - KNUST

3.5.2 Source of Materials

Freshly harvested pork (thigh muscles) and fat were purchased from the Kumasi Abattoir Company Limited and stored in chiller for 24 hours before use. The spices/herbs and other ingredients were obtained from the Kejetia market- Kumasi.

3.5.3 Formulation of Frankfurter Sausages

Frankfurter sausages were manufactured using the materials shown in the Table1 below

Table 1: Quantity of Ingredients used in the Frankfurter Sausage Formulations

Ingredient	% Composition	Quantity (g)
Lean Pork	70	2800
Fat	8	320
Curing Salt	1.2	48
Ice Cubes (Water)	18. 25	730
Essential oil	0.05	2
Phosphate	0.3	12
Spices	2.20	88
Total	**100**	**4000 (4kg)**

Note*: Spice mix included red pepper = 30g, onion = 25g, nutmeg = 20g, and garlic = 13g*

Chilled pork obtained from the muscles of the thigh were bought from the Kumasi Abattoir Company Limited and transported in chest freezer to the Department of Animal Science, KNUST. Each batch of sample was minced through a 5.0 mm mesh sieve and then ground together with the other ingredients to finer emulsion using the bowl cutter. A manual stuffer was used to stuff the emulsion into already prepared casing (small intestines) and linked at 10cm interval to form the frankfurter type sausages. They were then smoked in a smoke chamber for three hours and scalded at 70 °C until the internal temperature of 65 °C was achieved. Cooked products were allowed to cool down and kept frozen at -18 °C for further studies.

3.6 Evaluation of Sausages for Physico-chemical Properties

3.6.1 Cooking Yield/Loss

The final weight of products was expressed as a percentage of fresh weight to obtain the cooking yield (CY) and cooking loss (CL) calculated as (100 - %CY).

3.6.2 Proximate Composition of Products

Chemical composition such as crude protein, ash, moisture, fat and crude fibre and carbohydrate contents (%) were determined by the AOAC, (2000) methods. Protein determination involved a Kjeldahl assay (Nx6.25). Fat was determined by extracting samples in a Soxhlet apparatus using petroleum ether as a solvent. Moisture was quantified by oven-drying 5.0g samples at 105 °C. Ash was determined after incineration in a furnace at 550 °C. The carbohydrate content was calculated by computing the difference.

3.6.3 pH

The pH of fresh and cooked products was determined using a Mettler Toledo pH meter (FE20; GB/T111165). This was done for the cooked products during the storage periods of 7, 14 and 21days.

3.7 Evaluation of Sausages for Keeping Quality

3.7.1 Estimation of Antioxidant Activity in Products

Determination of the Free Radical Scavenging Activity was carried out by the 1, 1Diphenyl-2-picrylhydrazyl Free-Radical Scavenging Assay. Scavenging activities of the essential oils (in sausage products) on the stable free radical DPPH were assayed using the modified Blois‘ method (Wedlock, 2007), in which the bleaching rate of DPPH was monitored at a characteristic wavelength in the presence of the sample. A volume of 0.1 mL of an aqueous dilution of the products was mixed with 0.5 mL of a 500 µM DPPH solution in absolute ethanol and 0.4 mL of a 0.1M Tris-ClH buffer at pH 7.4. The mixture was kept for 20 min in darkness, and the absorbance was read at 517 nm using a Nanodrop (*ND 1000*) spectrophotometer in UV-Vis mode. The percentage of decrease of DPPH bleaching was calculated by measuring the absorbance of the sample and applying the following equation:

$$\textbf{\% of Inhibition} = [1 - (A_s/A_0)] \times 100,$$

Where $\mathbf{A_s}$ is the absorbance of sample (i.e., extracts), and $\mathbf{A_0}$ is the absorbance of the DPPH solution.

The antioxidant potential of the products was quantified by reference to a Trolox standard calibration curve. Trolox (6-hydroxy-2, 5, 7, 8 - tetramethylchroman2carboxylic acid) is a synthetic water soluble analogue of vitamin E that displays antioxidant activity. The Trolox solution was prepared using a 1.5mM Trolox stock solution. A standard curve was prepared using a control plus 5 different concentrations of Trolox/μM (0.135, 0.27, 0.405, 0.45, and 0.675) and absorbance measured and recorded. Results were then analyzed using Microsoft Excel XP Professional in order to create a relevant graph.

3.7.2 Free Fatty Acids (FFA)

A known weight (grams) of sample was weighed into a 250 mL Erlenmeyer flask to the nearest 0.01g. A volume of 50mL of neutralized ethanol was added. The resulting mixture was heated over a hot plate while swirling to completely dissolve the sample. Two drops of Phenolphthalein Indicator was added and solution titrated against 0.1N NaOH solution until the appearance of first permanent pink colour. Titre value was recorded. The %FFA was calculated as given below;

$$\%FFA = \frac{Titre\ value \times normality\ of\ NaOH \times M}{Weight\ of\ Sample}$$

Where M = 28.2 as Oleic Acid fraction for seed oils (AOCS, 2006).

3.7.3 Estimation of Microbial Load

Samples of all treatments were subjected to microbial count analysis following the procedure described by Omorodion and Odu, (2014). Ten (10) g of each sample was ground using stomacher and homogenized samples dispensed into a prepared 9ml of 0.1% peptone water and shaken for a homogenous mixture. Five- fold serial dilutions were used to prepare culture plates by the pour plate method. A 0.1ml of the 10^{-4} and 10^{-5} dilutions of each sample were pipetted out and pour plated using Nutrient agar (NA), MacConkey agar (MCA), Bismuth Sulphite Agar, Mannitol Salt Agar and Cetrimide Agar for total aerobic plate counts, total coliform (*Escherichia coli*) counts, total *Salmonella typhi* counts, total *Staphylococcus aureus* and total *Pseudomonas aeruginosa* count respectively. The plates were incubated at 37^0C for

24 - 48 hours. These were checked along the storage length at 7, 14 and 21 days. Colony forming units (CFU) for the various tests were counted and reported using the formulae as described by FAO, (2007) and APHA, (1993) and stated by Maturin and Peeler, (2001).

For plates with 25-250 CFU, **N =** $\frac{\sum c}{[(1xn_1)+(0.1xn_2)]xd}$; where

N = Number of colonies per ml or g of product $\sum$ C =

Sum of all colonies on all plates counted n_1 = Number

of plates in first dilution counted n_2 = Number of

plates in second dilution counted d = Dilution from

which the first counts were obtained

When plates from both dilutions yielded fewer than 25 CFU each, the actual plate count was recorded but the count was reported as less than **25 × 1/*d***; where ***d*** is the dilution factor for the dilution from which the first counts were obtained. However, when no growth was found on the plate, it was reported as less than 1 times the corresponding dilution used. The values obtained were then converted to **$\log_{10}$CFU/g.**

3.8 Sensory Evaluation

Pieces of coded hot cooked samples of the products were served to forty-five semitrained taste panelists to evaluate the sensory characteristics of the products. The products were coded with three - digits random numbers to ensure uniform and independent sampling. Taste panelists were provided with water to rinse the mouth between different treatments tasting to nullify any carry-on effects from one treatment to the other during the sensory testing. Attributes evaluated were colour/appearance, aroma (odour), taste, juiciness, tenderness, and overall acceptability of the products. A 9-point hedonic scale ranging from extremely bad (1) to extremely good (9) was used for the assessment.

3.8.1 Statistical Analysis

All data generated were subjected to one-way analysis of variance (ANOVA) in a completely randomized design (CRD) using SPSS version 16.0 for Windows (2007).

Significant differences between treatment means were determined by Duncan‘s test of homogeneity at 5% (Akwetey *et al*., 2012).

CHAPTER FOUR

4.0 RESULTS

Study1 Phase 1

4.1. Assessment of Spice/Herb Use in Kumasi Metropolis

4.1.1 Questionnaires Administered to Respondents

All questionnaires administered to consumers/domestic users and processors/vendors of meat products were retrieved.

4.2 Consumers/Domestic Users Response

4.2.1 The Demographic Characteristics of Respondents (Consumers/Domestic Users)

The demographic characteristics of consumers/domestic users of spices/herb and meat products interviewed for the purpose of this work are shown in Tables 2a, 2b and 2c. Out of 200 respondents, 25% were males and 75% were females. Most were below the age of 46years with just 18.5% aged 46year or above. Majority of the respondents (49%) were married while 40% were single, 6% divorced and 5% widowed.

The study identified 19 tribes across the 10 sub - metros of Kumasi as shown in Table 2b. Majority of the respondents were Akan (69.5%) with the rest of the 9 tribes forming 30.5%. The respondents were mostly Christians (75%). There were 23% Muslims while 2% were afiliated to other forms of religion. The study also showed that 54% respondents have household size less than 4, 28% respondents have household size between 4 and 6 inclusive while 18% respondents have household size of six (6) or more. Information gathered on the level of education indicated that 25%, 35%, 32.5% and 7.5% respondents had basic, secondary, tertiary and no formal education respectively.

Table 2a: Gender, Age and Marital Status of Consumers/Domestic Users of Spice/Herb in Kumasi Metropolis

Parameter	SUB - METRO											χ^2) Chi Square (≤ *p* *0.05*
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** (%)	**Kwadaso** No. No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No.(%)	**Tafo/ Pankrono** No. (%)	**Total** No. (%)	
Gender												
Male	9 (4.5)	9(4.5)	5 (2.5)	3(1.5)	2(1)	3(1.5)	10(5)	2(1)	5(2.5)	2(1)	**50**(25)	
Female	11 5.5)	11(5.5)	15(7.5)	17(8.5)	18(9)	17(8.5)	10(5)	18(9)	15(7.5)	18(9)	150(75)	24.5,
Total Respondents	**20 (10)**	**20 (10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	*p = 0.004*
Age(years)												
≤ 25												
26-35	3(1.5)	6(3)	2(1)	1(0.5)	4(2)	8(4)	6(3)	9(4.5)	4(2)	10(5)	53 (26.5)	
36-45	9(4.5)	5(2.5)	3(1.5)	5(2.5)	6(3)	5(2.5)	5(2.5)	5(2.5)	9(4.5)	5(2.5)	57(28.5)	
46--50	5(2.5)	3 (1.5)	11(5.5)	5(2.5)	7(3)	-	7(3.5)	2(1)	7(3.5)	5(2.5)	53(26.5)	
>50	1(0.5)	5(2.5)	3(1.5)	3(1.5)	1(0.5)	4(2)	2(1)	3(1.5)	-	-	22(11)	
total resp.	1(0.5)	1(0.5)	1(0.5)	6(3)	2(1)	3(1.5)	-	1(0.5)	-	-	15 (7.5)	71.8,
	20(10)	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	*p = 0.000*
Marital Status												
Single	8(4)	9(4.5)	5(2.5)	4(2)	2(1)	10(5)	10(5)	10(5)	10(5)	11(5.5)	80(40)	
Married	12(6)	7(3.5)	9(4.5)	12(6)	17(8.5)	7(3.5)	9(4.5)	9(4.5)	10(5)	8(4)	98 (49)	
Divorced	-	2(1)	5(2.5)	1(0.5)	-	-	1(0.5)	1(0.5)	-	1(0.5)	12 (6)	
Widowed	-	2(1)	1(0.5)	3(1.5)	1(0.5)	3(1.5)	-	-	-	-	10(5)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	54.1, *p = 0.001*

Note: total no. of respondents from the entire metropolis = 200 i.e. 100% *Dash (-) = no data obtained*

No. of respondents from each sub metro = 20 ie 10%

Table 2b: Tribe of Consumers/Domestic Users of Spice/Herb in Kumasi Metropolis

TRIBE	SUB - METRO											sc
	Asawase	**Asokwa**	**Bantama**	**Kwadaso**	**Manhyia**	**Nhyiaeso**	**Oforikrom**	**Suame**	**Subin**	**Tafo/Pankrono**	**Total**	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	P
Akan					16(8)	19(9.5)	11(5.5)	18(9)				

C

	13(6.5)	14(7)	16(8)	2(1)	-		-	4(2)	-		14(7)	139(69.5)	
Ga	1(0.5)	-	-	1(0.5)		1(0.5)		-	1(0.5)	1(0.5)	1(0.5)	7(3.5)	
Ewe	1(0.5)	-	1(0.5)	2(1)	-		-	-	-		1(0.5)	8(4)	
Frafra	2(1)	1(0.5)	-	-		1(0.5)		-	1(0.5)	-	-	3(1.5)	
Hausa	-	-	-	-		1(0.5)		-	-	-	-	2(1)	
Gonja	1(0.5)	1(0.5)	-	-	-		-	-	-		-	3(1.5)	
Dagaati	-	-	1(0.5)	3(1.5)	-		-	-	-		-	5(2.5)	
Dagbani	-	3(1.5)	1(0.5)	-	-		-	-	-		-	4(2)	
Mosie	-	-	-	3(1.5)		1(0.5)		-	1(0.5)	-	-	3(1.5)	
Bisa	-	-	-	3(1.5)							-	5(2.5)	2.
	-	-	-	2(1)							-	3(1.5)	
	-	-	-	1(0.5)							-	1(0.5)	P=
	-	-	-	1(0.5)							-	1(0.5)	
	1(0.5)	-	-	-							1(0.5)	4(2)	
	-	-	-	-							-	2(1)	
	-	1(0.5)	-	-							310.5)	4(2)	
	-	-	-	-							-	1(0.5)	
	1(0.5)	-	-	-							-	2(1)	
	-	-	1(0.5)	2(1)							-	3(1.5)	
Bulisa						-		-	1(0.5)	-			
Busanga						-		-	-	-			
Kasim						-		-	-	-			
Nzema						-		-	-	1(0.5)			
Kusasi						-		-	-	-			

Note*: Total No. of respondents from the entire metropolis = 200 i.e. 100%;*

No. of respondents from each sub metro = 20 i.e. 10%

Baasare					-	-	-	-			
Dagomba					-	1(0.5)	-	-			
Gurushi					-	-	1(0.5)	-			
Waale					-	-	-	-			
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**

Dash (-) = no data obtained

Table 2c: Religion, Household Size, Level of Education and Occupation of Consumers/Domestic Users of Spice/Herb in Kumasi Metropolis

Parameter	Sub- metro											χ^2) Chi square (p≤ 0.05
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No. (%)	**Tafo/Pankrono** N	**Total** No. (%)	
Religion												
Christianity	14(7)	9(4.5)	15(7.5)	7(3.5)	14(7)	20(10)	15(7.5)	20(10)	17(8.5)	19(9.5)	150(75)	
Islamic	5(2.5)	10(5)	5(2.5)	13(6.5)	5(2.5)	-	4(2)	-	3(1.5)	1(0.5)	46(23)	
Others	1(0.5)	1(0.5)	-	-	1(0.5)	-	1(0.5)	-	-	-	4(2)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	51.9, *p = 0.000*
Household Size												
<4	10(5)	13(6.5)	11(5.5)	4(2)	5(2.5)	17(8.5)	8(4)	8(4)	18(9)	14(7)	108(54)	
From 4- 6	7(3.5)	6(3)	4(2)	9(4.5)	9(4.5)	3(1.5)	6(3)	7(3.5)	1(0.5)	4(2)	56(28)	
>6	3(1.5)	1(0.5)	5(2.5)	7(3.5)	6(3)	-	6(3)	5(2.5)	1(0.5)	2(1)	36(18)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	45.1, *p = 0.000*

Level of Education												
Basic	1(0.5)	8(4)	3(1.5)	6(3)	7(3.5)	15(7.5)	1(0.5)	3(1.5)	4(2)	2(1)	50(25)	
Secondary	9(4.5)	4(2)	4(2)	7()3.5	7(3.5)	2(1)	6(3)	8(4)	8(4)	15(7.5)	70(35)	
Tertiary	9(4.5)	5(2.5)	12(6)	3(1.5)	4(2)	3(1.5)	10(5)	9(4.5)	7(3.5)	3(1.5)	65(32.5)	
No Formal	1(0.5)	3(1.5)	1(0.5)	4(2)	2(1)	-	3(1.5)	-	1(0.5)	-	15(7.5)	76.9, *p*
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	*= 0.000*
Occupation												
Civil service	7(3.5)	1(0.5)	4(2)	4(2)	3(1.5)	1(0.5)	5(2.5)	3(1.5)	2(1)	6(3)	36(18)	
Business (trade)	6(3)	12(6)	13(6.5)	12(6)	9(4.5)	11(5.5)	10(5)	8(4)	17(8.5)	14(7)	112(56)	
Others	7(3.5)	7(3.5)	3(1.5)	4(2)	8(4)	8(4)	5(2.5	9(4.5)	1(0.5)	-	52(26)	49.2, *p*
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	*= 0.006*

Dash (-) = no data obtained

Note*: Total No. of respondents from the entire metropolis = 200 i.e. 100%;*

No. of respondents from each sub metro = 20 i.e. 10%

The occupations of these respondents showed that 18% were civil servants, 56% engaged in business activities while 26% were involved in other forms of occupation. Statistically, all the chi square analysis showed that all the personal data were in significant ($p < 0.05$) association with respect to respondents' locations.

4.2.2 Spices/Herbs and their Preferred Forms Commonly Used in Meat/Food by Consumers/Domestic Users in Kumasi Metropolis The study showed that the respondents are multiple users of spices/herbs as shown in Table 3. Ginger, onion and chili pepper were commonly used by 72%, 38% and 32% respondents respectively. Negro pepper and prekese were frequently used by 44.5% and 41% respondents respectively. The use of ginger, chili pepper, negro pepper and prekese were statistically significant ($p<0.05$) in association with the respondents of the various sub-metros while onion use was not in association ($p>0.05$) with the locations of these respondents.

The preferred form of spices/herbs used by the consumers/domestic users are also reported in Table 4. Fifty-seven (57) % respondents preferred spices in powder form while 51% liked it in whole form (unprocessed natural from) with 28% going for the ground form. Statistically, the preference for powder, whole and paste forms of spices/herbs significantly ($p<0.05$) depended on the locations of the respondents.

Table 3. Spices/Herbs and the Preferred Forms Commonly Used by Consumers/Domestic Users in Kumasi Metropolis

PARAMETER	SUB METRO											Chi square (χ^2) $P \leq 0.05$
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No. (%)	**Tafo/ Pankrono** No. (%)	**Total** No. (%)	
Spice/Herb commonly used												
Ginger	15 (7.5)	17(8.5)	7(3.5)	10(5)	19(9.5)	14(7)	20(10)	16(8)	10(5)	16(8)	144(72)	20.6, *P = 0.015*
Onion	7(3.5)	2(1)	10(5)	11(5.5)	7(3.5)	2(1)	12(6)	6(3)	11(5.5)	8(4)	76(38)	11.1, *P = 0.267*
Chili pepper	10(5)	13(6.5)	12(6)	1(0.5)	3(1.5)	-	2(1)	8(4)	13(6.5)	3(1.5)	65 (32.5)	15.2, *P = 0.055*
Garlic	6(3)	4(2)	2(1)	6(3)	4(2)	3(1.5)	9(4.5)	5(2.5)	7(3.5)	11(5.5)	57(28.5)	18.4, *P = 0.031*
Aniseed	4(2)	2(1)	6(3)	2(1)	2(1)	13(6.5)	1(0.5)	4(2)	6(3)	1(0.5)	41(20.5)	7.6, *P = 0.475*
Nutmeg	2(1)	3(1.5)	4(2)	1(0.5)	2(1)	5(2.5)	2(1)	1(0.5)	7(3.5)	1(0.5)	28 (14)	9.9, *P = 0.355*
Negro pepper	9(4.5)	17(8.5)	15(7.5)	18(9)	10(5)	3(1.5)	5(2.5)	2(1)	3(1.5)	7(3.5)	89(44.5)	24.5, *P = 0.004*
Prekese	8(4)	12(6)	14(7)	17(8.5)	19(9.5)	4(2)	7(3.5)	-	-	4(2)	82(41)	25.3, *P = 0.001*
Calabash nutmeg	1(0.5)	2(1)	3(1.5)	1(0.5)	1(0.5)	3(1.5)	1(0.5)	-	-	2(1)	14(7)	11.03, *P = 0.137*
Grain of paradise	1(0.5)	-	1(0.5)	1(0.5)	1(0.5)	-	-	-	2(1)	3(1.5)	9(4.5)	6.1, *P = 0.296*
Cloves	1(0.5)	3(1.5)	3(1.5)	4(2)	1(0.5)	4(2)	1(0.5)	5(2.5)	3(1.5)	-	25(12.5)	9.5, *P = 0.302*
West African black pepper	-	4(2)	1(0.5)	2(1)	2(1)	-	-	1(0.5)	4(2)	1(0.5)	15(7.5)	2.6, *P= 0.854*
African locust bean	-	2(1)	2(1)	2(1)	1(0.5)	-	-	-	-	-	7(3.5)	-
Preferred Form of Spice/Herb Whole	11(5.5)	10(5)	16(8)	10(5)	14(7)	2(1)	10(6)	12(6)	13(6.5)	2(1)	102(51)	15.6, *p = 0.076*
Ground	4(2)	13(6.5)	3(1.5)	4(2)	1(0.5)	-	12(6)	5(2.5)	2(1)	12(6)	56(28)	5.3, *p = 0.724*
Paste	2(1)	8(4)	1(0.5)	3(1.5)	2(1)	7(3.5)	1(0.5)	6(3)	-	8(4)	38(19)	16.7, *p = 0.034*
Granular	5(2.5)	6(3)	1(0.5)	10(5)	-	8(4)	-	-	1(0.5)	2(1)	33(16.5)	8.6, *p =0. 196*
Powder	7(3.5)	11(5.5)	12(6)	8(4)	7(3.5)	10(5)	13(6.5)	12(6)	17(8.5)	16(8)	114(57)	20.5, *p = 0.015*
Others	-	4(2)	2(1)	2(1)	3(1.5)	2(1)	1(0.5)	-	-	-	14(7)	8.06, *p = 0.153*

Total number of respondents for each specific spice/herb or its preferred form = 200 (100%). Total number of respondent in each sub metro = 20(10%).
Chi square analysis** is possible for observations with **five** or **more count only.

4.2.3 Common Uses of Spices/Herbs in Meat/Food Products by Consumers/Domestic Users in Kumasi Metropolis

The common uses of spices/herbs by the consumers/domestic users are reported in Table 4. Among the common uses of the spices/herbs by the consumers/domestic users were stew - 77%, soup -74%, sausages -57.5%, kebab - 49.5%, jollof - 45% and meat loaf - 26%. Twenty-four percentage (24%) use spices/herbs for other forms food preparations, 8.5% use spices/herbs for barbecue, 8% use spices/herbs for burgers and 3.5% use spices/herbs for other forms of meat products. Statistically, the use of spices/herbs for preparing stew, sausages, meat loaf and other meals were significantly ($p<0.05$) associated with the sub-metros of the respondents but not significant ($p>0.05$) between spice use for soup, jollof, burgers, barbecue, kebab or other meat products and sub metros of respondents.

4.2.4 Income of Consumers/Domestic Users and How Much they Spend on Spice/Herb in the Metropolis

Information on the monthly income of consumers/domestic users of meat/food products is shown in Table 4. The investigation revealed that - 44%, 26.5%, 19% and 8.5% receive between GH¢100 - GH¢500, less than GH¢100, between GH¢500 - GH¢1000 and above GH¢1000 respectively. The income received by the respondents was not significantly ($p>0.05$) associated with the locations of these respondents.

The monthly expenses on spice/herbs by consumers/domestic users in study area are also presented in Table 4. From study, 47.5%, 38.5%, 10.5% and 1.5% respondents spend GH¢1- GH¢5, GH¢6 - GH¢10, GH¢11- GH¢50 and above GH¢50 respectively on spices every month. The observation was statistically significant ($p<0.05$) for expenditure of GH¢1- GH¢5 with respcet to location of respondents but not significant ($p>0.05$) for the other ranges.

4.2.5 Health Concerns and Misgivings on Spice/Herbs Usage by Consumers/Domestic Users of Meat/Food Products

In respect of health concerns associated with the use of spices/herbs in meat/food products, the study showed, as presented in Table 5 that 78.5% of respondents did not think spice/herbs pose health problems while 21.5% thought spice/herbs usage pose health threat. Statistically, the health responses did not significantly ($P>0.05$) depend on the locations of the respondents in this study.

Some misgivings noted included; ill health, not good for food preparation, change of taste of food, expensive, difficulty to obtain and difficulty in preparing spices in whole form. These misgivings are presented in Table 5 as B, C, D, E, F, and G. A implies no misgiving. The study showed that 68.5% respondents did not have any misgiving on the use of spices/herbs in meat and food products. Also, 21.5% thought spices/herbs can cause ill health in individuals who use them, 2% thought spices/herbs are not good for food preparation, 5% said spice/herb can change the usual taste of food product, 0.5% were concerned with its cost (expensive), 1.5% complained about the difficulty in obtaining some of these spices while 1% were not comfortable with the difficulty one goes through to prepare spices/herbs in the whole form to the preferred usable form.

Metropolis

Table 4. Common Uses of Spices/Herbs, Monthly Income and Expenses on Spice/Herbs by Consumers/Domestic Users in Kumasi

PARAMETER	SUB METRO											Chi square (χ^2)
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No. (%)	**Tafo/ Pankrono** No. (%)	**Total** No. (%)	$P \leq 0.05$
Common uses of Spice/Herb												
Stew	15(7.5)	15(7.5)	12(6)	13(6.5)	17(8.5)	12(6)	17(8.5)	17(8.5)	16(8)	20(10)	154(77)	30.5, $P = 0.000$
Soup	15(7.5)	13(6.5)	12(6)	17(8.5)	17(8.5)	12(6)	10(5)	14(7)	18(9)	20(10)	148(74)	14.7, $P = 0.100$
Jollof	7(3.5)	10(5)	7(3.5)	10(5)	7(3.5)	3(1.5)	10(5)	7(3.5)	10(5)	19(9.5)	90(45)	14.9, $P = 0.094$
Other meals	4(2)	4(2)	-	2(1)	1(0.5)	-	7(3.5)	11(5.5)	5(2.5)	2(1)	36(18)	13.9, $P = 0.053$
Sausages	11(5.5)	15(7.5)	6(3)	7(8.5)	15(7.5)	3(1.5)	10(5)	12(6)	17(8.5)	19(9.5)	115(57.5)	19.5, $P = 0.021$
Burgers	-	-	3(1.5)	2(1)	-	-	-	1(0.5)	2(1)	8(4)	16(8)	1.06, $P = 0.900$
Meat loaf	4(2)	1(0.5)	10(5)	4(2)	5(2.5)	11(5.5)	11(5.5)	2(1)	3(1.5)	1(0.5)	52(26)	17.3, $P = 0.044$
Barbecue	-	3(1.5)	3(1.5)	2(1)	1(0.5)	4(2)	-	2(1)	1(0.5)	1(0.5)	17(8.5)	4.6, $P = 0.714$
Khebab	14(7)	17(8.5)	3(1.5)	8(4)	8(4)	4(2)	9(4.5)	13(6.5)	12(6)	12(6)	99(49.5)	13.5, $P = 0.139$
Other meat products	1(0.5)	3(1.5)	-	1(0.5)	-	-	-	2(1)	-	-	7(3.5)	1.6, $P = 0.670$

8(4)	-	3(1.5)	1(0.5)	9(4.5)	6(3)	6(3)	7(3.5)		
7(3.5)	5(2.5)	8(4)	13(6.5)	8(4)	10(5)	8(4)	11(5.5)		
-	7(3.5)	8(4)	5(2.5)	2(1)	3(1.5)	4(1)	2(1)		
2(1)	8(4)	1(0.5)	1(0.5)	1(0.5)	1(0.5)	2(1)	-	-	
3(1.5)	-	-	-	-	-	-	-	-	

Monthly income (GH¢)				
< 100	2(1)	11(5.5)	53(26.5)	11.7, $p = 0.167$
100-500	10(5)	8(4)	88(44)	10.1, $p = 0.340$
500-1000	6(3)	1(0.5)	38(19)	14.9, $p = 0.060$
Above 1000	1(0.5)		17(8.5)	5.1, $p = 0.644$
No comment	1(0.5)		4(2)	-

observations with ***fiv***

Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	
Monthly expenses on spice/herb (GH¢) 1-5	9(4.5)	6(3)	16(8)	6(3)	5(2.5)	13(6.5)	12(6)	9(4.5)	11(5.5)	8(4)	95(47.5)	20.0, *p = 0.018*
6-10	8(4)	7(3.5)	3(1.5)	10(5)	9(4.5)	6(3)	7(3.5)	9(4.5)	7(3.5)	11(5.5)	77(38.5)	14.3, *p = 0.113*
11-50	2(1)	4(2)	1(0.5)	3(1.5)	4(2)	1(0.5)	1(0.5)	2(1)	2(1)	1(0.5)	21 (10.5)	10.8, *p = 0.113*
Above 50	-	-	-	1(0.5)	2(1)	-	-	-	-	-	3(1.5)	-
No Comment	1(0.5)	3(1.5)	-	-	-	-	-	-	-		4(2)	-
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	

*Total number of respondents for each specific use of spice/herb = 200 (100%). Total number of respondent in each sub metro = 20(10%)****. Chi square analysis*** *is possible for*

Table 5. Health concerns and misgivings on the use of Spices/herbs, by Consumers/Domestic users in Kumasi metropolis

PARAMETER	SUB METRO											Chi square (χ^2) $P \leq 0.05$
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No. (%)	**Tafo/ Pankrono** No. (%)	**Total** No. (%)	
HEALTH CONCERNS ON SPICE/HERB USE												
Spice/herb ***cause ill health***	4(2)	2(1)	1(0.5)	1(0.5)	2(1)	4(2)	12(6)	10(5)	6(3)	1(0.5)	43(21.5)	14.7, *P = 0.101*
Spice/***herb does not cause ill health***	16(8)	18(9)	19(9.5)	19(9.5)	18(9)	16(8)	8(4)	10(5)	14(7)	19(9.5)	157(78.5)	15.7, *P = 0.740*
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	

A- No misgivings

Misgivings on spice/herb usage A	16(8)	17(8.5)	19(9.5)	17(8.5)	11(5.5)	13(6.5)	8(4)	8(4)	10(5)	18(9)	137(68.5)	17.5, *p = 0.041*
B	4(2)	2(1)	1(0.5)	1(0.5)	2(1)	4(2)	12(6)	10(5)	6(3)	1(0.5)	43(21.5)	14.7, *P = 0.101*
C	-	-	-	-	3(1.5)	-	-	-	-	1(0.5)	4(2)	-
D	-	1(0.5)	-	1(0.5)	2(1)	2(1)	-	-	4(2)	-	10(5)	4.6, P = 0.098
E	-	-	-	1(0.5)	-	-	-	-	-	-	1(0.5)	-
F	-	-	-	-	2(1)	1(0.5)	-	-	-	-	3(1.5)	-
G	-	-	-	-	-	-	-	2(1)	-	-	2(1)	-
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	

D - Excess change food taste

E- Expensive

F- Some are difficult to obtain;

G- Difficulty in using spice in whole form

B - Can cause ill health

C - Not good for food preparation

Chi square analysis *is possible for observations with* ***five*** *or* ***more count only.***

4.3.0 Processors/Vendors Response

4.3.1 The Demographic Characteristics of the Respondents (Processors/Vendors) in Kumasi Metropolis

The demographic characteristics of 200 processors/vendors of this study are shown in Table 6a, 6b and 6c. From the survey, 42.5% males and 57.5% females who engage in meat processing or sale of meat products were interviewed across the ten sub-metros of Kumasi metropolis. Gender of respondents did not significantly ($p>0.05$) depend on the locations of these respondents.

The study shows that 17.5% respondents were less or equal to 25years of age, 43% were between the ages of 26-35years, 28.5% were between the ages 36-45years, 6% were between the ages of 45-50years and 3.5% were above 50years of age. The ages of the respondents were significantly ($p<0.05$) dependent on the locations of this study.

The majority (57.5%) of the respondents were married, 33% were singles, 6% were divorced while 3.5% were widowed. Statistical analysis also indicated that the marital status of the respondents was not significantly ($p>0.05$) associated with locations of the study.

There were 56% Akan-speaking people, 11% Frafra, 6.5% Ewe, 6% Ga, 4.5% Hausa and 3% Gonja. The rest consisted of Dagaati, Mosie, Dagomba, Busanga, Bulisa, Gurishi, Waale, Bisa, Kasim, Nzema, Baasare and Sisala. In all, 18 tribes were identified across the 10 sub metros of the city. The tribe of residents was associated significantly ($p<0.05$) with the places of residence.

Among the processors/vendors, 65% were Christians, 31.5% were Muslims while 3.5% were afiliated to other forms of religion. The religious background of the respondents was significantly ($p<0.05$) associated with the locations of the respondents.

Information on household size showed that 48% respondents have less than 4 household size, 34% have household size between 4 and 6 inclusive while 18% have household size above six (6). The household size of the respondents does not depend on the sub-metros of these respondents significantly ($p>0.05$).

The education background check of the respondents showed that 33%, 35.5%, 13.5% and 18% had basic education, secondary education, tertiary education and no formal education respectively. Statistically, the educational background information was significantly ($p<0.05$) associated with the locations of these respondents.

Apart from the processing/selling of meat/meat products, the respondents were also found to engage in other occupational activities. Out of 200 respondents, 49% do business/trading, 39% are engaged in other occupations while 12% are civil servants. The additional occupation of these respondents was significantly ($p<0.05$) associated with their locations.

No. of respondents from each sub metr

Table 6a: Gender, Age and Marital Status of Processors/Vendors of Meat Products in Kumasi Metropolis

Parameter	SUB - METRO											Chi Square (χ^2) $p \leq 0.05$
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No.(%)	**Tafo/ Pankrono** No. (%)	**Total** No. (%)	
Gender												
Male	5(2.5)	8(4)	9(4.5)	8(4)	6(3)	7(3.5)	10(5)	11(5.5)	11(5.5)	10(5)	**85**(42.5)	
Female	15(7.5)	12(6)	11(5.5)	12(6)	14(7)	13(6.5)	10(5)	9(4.5)	9(4.5)	10(5)	115(57.5)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	7.9, $p = 0.347$
Age(years)												
≤ 25	2(1)	3(1.5)	9(4.5)	2(1)	1(0.5)	6(3)	4(2)	2(1)	-	6(3)	35(17.5)	
26-35	12(6)	7(3.5)	5(2.5)	11(5.5)	11(5.5)	8(4)	3(1.5)	11(5.5)	11(5.5)	7(3.5)	86(43)	
36-45	5(2.5)	7(3.5) 2(1)	5(2.5)	5(2.5)	7(3.5)	5(2.5)	5(2.5)	7(3.5)	8(4)	3(1.5)	57(28.5)	
46--50	1(0.5)	1(0.5)	1(0.5)	-	-	1(0.5)	2(1)	-	1(0.5)	4(2)	12(6)	
>50	-	**20(10)**	-	2(1)	1(0.5)	-	6	-	-	-	10(5)	
Total Respondents	**20(10)**		**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	76.245, $p = 0.000$
Marital Status												
Single	9(4.5)	7(3.5)	7(3.5)	8(4)	2(1)	8(4)	8(4)	4(2)	7(3.5)	6(3)	66(33)	
Married	10(5)	11(5.5)	8(4)	10(5)	16(8)	10(5)	10(5)	15(7.5)	12(6)	11(5.5)	115(57.5)	
Divorced	1(0.5)	1(0.5)	3(1.5)	-	2(1)	2(1)	2(1)	1(0.5)	-	2(1)	12(6)	
Widowed	-	1(0.5)	2(1)	2(1)	-	-	-	-	1(0.5)	1(0.5)	7(3.5)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	27.4, $p = 0.442$

Note: Total No. of respondents from the entire metropolis = 200 i.e. 100% *Dash (-) = no data obtained*

Table 6b: Tribe of Processors/Vendors of Meat Prodcuts in Kumasi Metropolis

TRIBE	SUB - METRO											Chi (χ^2) $P \leq 0.05$ Square
	Asawase No. (%)	**Asokwa No.(%)**	**Bantama No. (%)**	**Kwadaso No.(%)**	**Manhyia No. (%)**	**Nhyiaeso No.(%)**	**Oforikrom No. (%)**	**Suame No.(%)**	**Subin No. (%)**	**Tafo/Pankrono No.(%)**	**Total No. (%)**	
Akan	15(7.5)	10(5)	12(6)	9(4.5)	12(6)	16(8)	9(4.5)	8(4)	9(4.5)	12(6)	112(56)	
Ga	1(0.5)	1(0.5)	4(2)	-	1(0.5)	-	1(0.5)	-	3(1.5)	1(0.5)	12(6)	
Ewe	-	-	2(1)	2(1)	1(0.5)	-	3()1.5	-	3(1.5)	2(1)	13(6.5)	
Frafra	1(0.5)	2(1)	-	3(1.5)	-	-	5(2.5)	11(5.5)	-	-	22(11)	
Hausa	1(0.5)	-	-	-	1(0.5)	4(2)	-	1(0.5)	2(1)	-	9(4.5)	
Gonja	1(0.5)	1(0.5)	2(1)	-	-	-	-	-	2(1)	-	6(3)	
Dagaati	-	-	-	1(0.5)	-	-	-	-	1(0.5)	2(1)	4(2)	2.3,
Mosie	-	2(1)	-	1(0.5)	-	-	-	-	-	1(0.5)	4(2)	P = 0.000

Bisa	-	-	-	1(0.5)	-	-	-	-	-	-	1(0.5)

Bulisa	-	-	-	2(1)	-	-	-	-	-	-	2(1)
Busanga	1(0.5)	-	-	-	2(1)	-	-	-	-	-	3(1.5)
Kasim	-	1(0.5)	-	-	-	-	-	-	-	-	1(0.5)
Nzema	-	-	-	-	-	-	1(0.5)	-	-	-	1(0.5)
Sisala	-	-	-	-	-	-	1(0.5)	-	-	-	1(0.5)
Baasare	-	-	-	-	1(0.5)	-	-	-	-	-	1(0.5)
Dagomba	-	1(0.5)	-	-	1(0.5)	-	-	-	-	2(1)	4(2)
Gurushi	-	1(0.5)	-	1(0.5)	-	-	-	-	-	-	2(1)
Waale	-	1(0.5)	-	-	1(0.5)	-	-	-	-	-	2(1)
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**

Note: Total no. of respondents from the entire metropolis = 200 i.e. 100% *Dash (-) = no data obtained*

No. of Respondents fromeach sub metro = 20 i.e. 10%

Table 6c: Religion, Household Size, Level of Education and Occupation of Processors/Vendors of Meat Products in Kumasi Metropolis

parameter	SUB - METRO											Chi (χ^2) $P \leq 0.05$ Square
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No.(%)	**Tafo/ Pankrono** No. (%)	**Total** No. (%)	
Christianity	16(8)	10(5)	14(7)	11(5.5)	11(5.5)	14(7)	16(8)	11(5.5)	12(6)	15(7.5)	130 (65)	
Islamic	4(2)	10(5)	3(1.5)	9(4.5)	9(4.5)	6(3)	2(1)	9(4.5)	7(3.5)	4(2)	63(31.5)	
Others	-	-	3(1.5)	-	-	-	2(1)	-	1(0.5)	1(0.5)	7(3.5)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200 (100)**	30.05, *p = 0.037*
Household size <4	11(5.5)	11(5.5)	11(5.5)	11(5.5)	6(3)	9(4.5)	94.5)	10	11(5.5)	7(3.5)	96(48)	
from 4- 6	5(2.5)	3(1.5)	7(3.5)	7(3.5)	4(2)	9(4.5)	8(4)	7(3.5)	8(4)	10(5)	68(34)	
>6	4(2)	6(3)	2(1)	2(1)	10(5)	2(1)	3(1.5)	3(1.5)	1(0.5)	3(1.5)	36(18)	
Total Resspondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	26.9, *p = 0.081*
Level of education basic	11(5.5)	8(4)	3(1.5)	5(2.5)	12(6)	12(6)	1(0.5)	7(3.5)	3(1.5)	4(2)	66(33)	
secondary	4(2)	6(3)	8(4)	10(5)	6(3)	6(3)	7(3.5)	12(6)	7(3.5)	8(4)	71(35.5)	
Tertiary no	3(1.5)	-	6(3)	1(0.5)	2(1)	2(1)	6(3)	1(0.5)	3(1.5)	5(2.5)	27(13.5)	
formal	2(1)	6(3)	3(1.5)	4(2)	-	-	6(3)	-	7(3.5)	3(1.5)	36(18)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	84.9, *p = 0.000*
Occupation Civil service	4(2)	2(1)	4(2)	1(0.5)	-	2(1)	1(0.5)	1(0.5)	1(0.5)	6(3)	24(12)	
Business (trade)	11(5.5)	16	13(6.5)	2(1)	14(7)	3(1.5)	6(3)	11(5.5)	13(6.5)	9(4.5)	98 (46)	
others	5(2.5)	2(1)	3(1.5)	17(8.5)	6(3)	15(7.5)	11(5.5)	8(4)	6(3)	5(2.5)	78(36)	
Total Respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	88.8, *p = 0.000*

***Note**: Total No. of respondents from the entire me*

Dash (-)= no data obtained No.

respondents from each sub metro = 20 i.e. 10%'

4.3.2 Spices Commonly Used and their Preferred Forms in Meat Products by Processors/Vendors of Meat Products in Kumasi Metropolis

The response from the 200 processors/vendors interviewed, as shown in Table 7, indicate that ginger, onion and chili pepper are mostly used by the respondents and recorded 82.5%, 67% and 64% respectively. More than half of respondents from each location used ginger quite often in meat products. Garlic, aniseed and nutmeg also recorded 44%, 35.5% and 29% usage respectively. From the study, 32.5%, 31.5%, 14.5% and18% respondents include Negro pepper, prekese, calabash nutmeg and Grains of paradise, respectively in their meat products. Cloves, West African black pepper and African locust bean were also employed in meat product preparations by 19.5%, 10.5% and 14.5% respondents respectively. The statistical analysis as shown in Table 8 shows that the frequency of use of the spices/herbs (Negro pepper, prekese and calabash nutmeg) use was significantly ($p<0.05$) dependent on the locations of the respondents. However, the frequency of use of the remaining spices/herbs was insignificant ($p>0.05$) in association with the locations of the respondents.

The preferred form of spices/herbs by the processors/vendors is also reported in Table 7. The respondents preferred spices/herbs in powder - 71.0%, whole (natural form) - 52.0%, ground (25%), paste (19.5%), granular (14.0%) forms while 5% were not sure of which form they preferred. Statistical analysis showed that the preferred form of spice/herb was insignificantly ($p>0.05$) associated with the locations of the respondents.

Metropolis

Table 7. Spices/Herbs and the Preferred Forms Commonly Used by Processors/Vendors of Meat Products in Kumasi

PARAMETER	SUB METRO											Chi square (χ^2) $P \leq 0.05$
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No. (%)	**Tafo/ Pankrono** No. (%)	**Total** No. (%)	
Spice/Herb commonly used												
Ginger	14(7)	14(7)	15(7.5)	17(8.5)	18(9)	17(8.5)	17(8.5)	19(9.5)	17(8.5)	17(8.5)	165(82.5)	7.4, *P = 0.594*
Onion	9(4.5)	13(6.5)	20(10)	13(6.5)	10(5)	2(1)	15(7.5)	17(8.5)	18(9)	17(8.5)	134(67)	6.9, *P = 0.652*
Chili pepper	9(4.5)	15(7.5)	16(8)	17(8.5)	4(2)	8(4)	17(8.5)	11(5.5)	17(8.5)	14(7)	128(64)	9.9, *P = 0.358*
Garlic	10(5)	4(2)	6(3)	8(4)	10(5)	3(1.5)	8(4)	14(7)	10(5)	13(6.5)	88(44)	7.1, *P = 0.631*
Aniseed	11(5.5)	6(3)	10(5)	4(2)	10(5)	5(2.5)	4(2)	9(4.5)	6(3)	1(0.5)	66(33)	8.1, *P = 0.520*
Nutmeg	10(5)	1(0.5)	6(3)	9(4.5)	3(1.5)	12(6)	2(1)	1(0.5)	5(2.5)	9(4.5)	58(29)	11.7, *P = 0.229*
Negro pepper	14(7)	17(8.5)	4(2)	7(3.5)	4(2)	2(1)	2(1)	4(2)	5(2.5)	6(3)	65(32.5)	18.5, *P = 0.030*
Prekese	11(5.5)	11(5.5)	1(0.5)	5(2.5)	8(4)	9(4.5)	8(4)	5(2.5)	2(1)	3(1.5)	63(31.5)	28.5, *P = 0.001*
Calabash nutmeg	4(2)	4(2)	4(2)	3(1.5)	-	2(1)	3(1.5)	3(1.5)	-	6(3)	29(14.5)	13.98, *P =0.051*
Grain of paradise	8(4)	4(2)	1(0.5)	2(1)	5(2.5)	3(1.5)	1(0.5)	-	3(1.5)	9(4.5)	36(18)	14.8, *P = 0.063*
Cloves	11(5.5)	2(1)	4(2)	4(2)	7(3.5)	9(4.5)	3(1.5)	5(2.5)	4(2)	2(1)	51(25.5)	12.7, *P = 00177*
West African black pepper	10(5)	1(0.5)	-	-	4(2)	-	2(1)	-	4(2)	-	21(10.5)	2.5, *P= 0.642*
African locust bean	3(1.5)	2(1)	-	6(3)	3(1.5)	3(1.5)	-	3(1.5)	2(1)	2(1)	29(14.5)	8.1, p = 0.323
Preferred Form of Spice/Herb												
Whole	8(4)	15(5.5)	19(9.5)	11(5.5)	9(4.5)	3(1.5)	8(4)	5(2.5)	12(6)	14(7)	104(52)	11.3, *p = 0.257*
Ground	4(2)	7(3.5)	13(6.5)	-	1(0.5)	2(1)	8(4)	-	4(2)	11(5.5)	50(25)	7.1, *p = 0.414*
Paste	2(1)	3(1.5)	1(0.5)	7(3.5)	-	6(3)	2(1)	6(3)	2(1)	10(5)	39(19.5)	10.01, *p=0.264*
Granular	2(1)	4(2)	5(2.5)	3(1.5)	1(0.5)	5(2.5)	-	-	1(0.5)	7(3.5)	28(14)	10.3, *p =0. 171*
Powder	10(5)	12(6)	5(2.5)	19(9.5)	11(5.5)	15(7.5)	14(7)	20(10)	20(10)	16(8)	142(71)	3.3, *p = 0.0952*
Others	2(1)	2(1)	-	-	1(0.5)	2(1)	-	-	-	3(1.5)	10(5)	4.4, *p = 0.349*

Total number of respondents for each specific spice/herb or it preferred form = 200 (100%). Total number of respondent in each sub metro = 20(10%).

4.3.3 Common Uses of Spices/Herbs by Processors/Vendors of Meat Products in Kumasi Metropolis

The common uses of spices/herbs by the processors/vendors are reported in Table 8. The study shows that the processors/vendors used spices/herbs more often in the preparation of kebab - 85.5%, sausages - 70.5%, barbecue - 19%, meat loaf - 18.5%, burgers - 15% while just 6.0% respondents used spices/herbs in other forms of meat products. The common use of spice/herb was not significantly ($p>0.05$) associated with locations of the respondents.

4.3.4 Production Cost of Meat Products in the Metropolis

The cost of producing a kg of meat product and the cost of spices/herbs needed for the product are presented in Table 8. The study showed that 33%, 57.5%, and 9.5% respondents could produce a kilogram of meat product at a cost of GH¢1- GH¢10, GH¢11- GH¢15 and above GH¢ 15 respectively. Costing the spice/herb used per kg meat product produced, it was revealed that, 43%, 40.5% and 16.5% respondents used spices/herbs worth GH¢1- GH¢5, GH¢6 - GH¢10, and GH¢11- GH¢15 respectively. Statistical analysis showed that the average cost of producing a kg of meat product and the cost of spices/herbs were insignificantly ($p>0.05$) dependent on the locations of the respondents.

Table 8. Common Uses of Spices/Herbs and Cost of Spice/Herbs in Meat Product by Meat Processors/Vendors in Kumasi Metropolis

PARAMETER	SUB METRO											Chi square(χ^2) $P \leq 0.05$
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No. (%)	**Tafo/Pankrono** No. (%)	**Total** No. (%)	
Common uses of Spice/Herb												
Sausages	11(5.5)	14(7)	14(7)	15(7.5)	16(8)	14(7)	11(5.5)	11(5.5)	18(9)	17(8.5)	141(70.5)	13.6, $P = 0.136$
Burgers	1(0.5)	1(0.5)	4(2)	-	6(3)	7(3.5)	1(0.5)	-	7(3.5)	3(1.5)	30(15)	4.6, $P = 0.706$
Meat loaf	6(3)	-	11(5.5)	3(1.5)	2(1)	7(35.5)	6(3)	-	-	-	37(18.5)	4.7, $P = 0.0576$
Barbecue	3(1.5)	1(0.5)	3(1.5)	8(4)	5(2.5)	10(5)	-	-	7(3.5)	1(0.5)	38(19)	8.1, $P = 0.326$
Khebab	12(6)	19(9.5)	19(9.5)	17(8.5)	16(8)	11(5.5)	18(9)	20(10)	19(9.5)	20(10)	171(85.5)	5.02, $P = 0.832$
Other meat products	6(3)	-	-	2(1)	1(0.5)	1(0.5)	-	-	-	-	12(6)	5.8, $P = 0.212$
Production cost /Kg meat product (GH¢)												
1-10	7(3.5)	6(3)	15(7.5)	1(0.5)	9(4.5)	3(1.5)	5(2.5)	1(0.5)	12(6)	7(3.5)	66(33)	11.7, $p = 0.721$
11- 15	12(6)	13(6.5)	5(2.5)	19(9.5)	10(5)	16(8)	15(7.5)	5(2.5)	8(4)	12(6)	115(57.5)	14.2, $p = 0.111$
> 15	1(0.5)	1(0.5)	-	-	1(0.5)	1(0.5)	-	14(7)	-	1(0.5)	19(9.5)	6.7, $p = 0.242$
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	
Cost of spice/herb /Kg meat product (GH¢)												
1-5	11(5.5)	5(2.5)	10(5)	8(4)	12(6)	6(3)	10(5)	7(3.5)	14(7)	3(1.5)	86(43)	13.8, p = 0.129
6-10	7(3.5)	7(3.5)	7(3.5)	10(5)	5(2.5)	8(4)	8(4)	10(5)	6(3)	13(6.5)	81(40.5)	14.2, p = 0.0132
11-15	2(1)	8(4)	3(1.5)	2(1)	3(1.5)	6(3)	2(1)	3(1.5)	4(2)	4(2)	33(16.5)	10.8, p = 0.129
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	

Total number of respondents for each specific use of spice/herb = 200 (100%). Total number of respondent in each sub metro = 20(10%)

4.3.5 Health Problems and Misgivings Associated with Spic/Herb Usage in the Metropolis

Investigations into the health related problems and misgivings associated with the use of spices/herbs in the metropolis are presented in Table 9. Out 200 processors/vendors interviewed 13% believed spice/herb usage can cause ill - health while 87% did not agree with them. All respondents from Asokwa and Bantama believed spices/herbs do not cause any ill health. However, respondents between 0.5% and 3% respondents across all sub metros believed spice/herb usage can cause health problems.

On specific health problems, 10 %, said spice/herb usage can cause heart diseases, 3% believed spice/herb usage could lead to allergy and stomach clumps while 87% reserved their comments. Those who believe that spice/herb use cause heart problems were mostly residents of Suame and other sub metros except Asawase and Asokwa where no respondent agreed to that assertion. Allergy and stomach clumps were believed to be associated with spice/herb use by few respondents from Manhyia, Nhyiaeso and Oforikrom.

Misgivings on the use of spices/herbs by processors/vendors from the metropolis showed 85% had no misgivings, 5.5% did not like the scent/smell of some of the spices, 2.5% were concerned with how difficult it is obtain some of the spices, 1% thought spice/herbs are expensive to buy, 1% believed it could cause heart problems, 1.5% talked about the cumbersome initial preparation for some spices while 3.5% believed excess use may change taste of food. Statistically, the health problems and misgivings associated with the use of spices/herb was not in significant ($p>0.05$) association with the locations of respondents in this study.

Table 9. Health Concerns and Misgivings on the Use of Spices/Herbs Meat Processors/Vendors Users in Kumasi Metropolis

PARAMETER	SUB METRO											Chi square (χ^2) $P \leq 0.05$
	Asawase No. (%)	**Asokwa** No. (%)	**Bantama** No. (%)	**Kwadaso** No. (%)	**Manhyia** No. (%)	**Nhyiaeso** No. (%)	**Oforikrom** No. (%)	**Suame** No. (%)	**Subin** No. (%)	**Tafo/Pankrono** No. (%)	**Total** No. (%)	
HEALTH CONCERNS ON SPICE/HERB USE												
Spice/herb ***cause ill health***	3(1.5)	-	-	3(1.5)	1(0.5)	3(1.5)	3(1.5)	6(3)	2(1)	5(2.5)	26(13)	2.5, *P = 0.931*
Spice/***herb does not cause ill health***	17(8.5)	20(10)	20(10)	17(8.5)	19(9.5)	17(8.5)	17(8.5)	14(7)	18(9)	15(7.5)	174(87)	3.4, *P = 0.844*
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	
Specific Health Problem												
Heart diseases	1(0.5)	-	-	2(1)	1(0.5)	3(1.5)	2(1)	6(3)	2(1)	3(1.5)	20(10)	4.4, p = 0.734
Allergy/stomach clumps	-	-	-	-	2(1)	2(1)	2(1)	-	-	-	6(3)	-
No comment	19	20	20	18	17	15	16	14	18	17	174(87)	9.3, p = 0.411
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	
Misgivings on spice/herb usage A	17(8.5)	19(9.5)	20(10)	18(9)	20(10)	11(5.5)	18(9)	18(9)	14(7)	15(7.5)	170(85)	9.2, *p = 0.423*
B	2(1)	-	-	2(1)	-	2(1)	2(1)	-	-	3(1.5)	11(5.5)	6.3, *P = 0.178*
C	1	-	-	-	-	3	-	-	-	1	5(2.5)	-
D	-	-	-	-	-	2	-	-	-	-	2(1)	-
E	-	-	-	-	-	2	-	-	-	-	2(1)	-
F	-	1	-	-	-	-	-	2	-	-	3(1.5)	-
G	-	-	-	-	-	-	-	-	6(3)	1(0.5)	7(3.5)	-
Total respondents	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**20(10)**	**200(100)**	

Note: A- No Misgivings; B- Dislike smell of some spice; C - Difficult to obtain some spices; D - Expensive; E - May give heart problems; F - Cumbersome initial preparations; G-Excess may change taste of food; ***Chi square analysis*** *is possible for observations with* ***five*** *or* ***more count only***

Study 1 Phase 2

4.4 Phytochemical Content and Functional Properties of Selected Spices/Herb

4.4.1 Screening and Quantification of Phytochemicals in the Spices/Herb From the survey conducted, three most frequently used spices with international reputation namely ginger, onion and chili pepper were selected alongside with two most frequently used indigenous spices (i.e.negro pepper and prekese) for further studies.

The presence and quantity of phytochemicals tested for in the various spices are shown in Table 10. Phenol was highest in chili pepper (Cp) - 1.667% and least in onion - 0.528%. Flavonoids content ranged from 3.045% in ginger to 0.704% in Negro pepper (NP), tannins- 0.609% (onion) to 0.447% in Negro pepper, alkaloids- 0.677% (Cp) to 0.059% ginger and saponins- 2.952% (ginger) to 0.652% (onion). Essential oils extracted from the spices were used in the manufacture of frankfurter sausages.

Table 10: Screening and Quantification of Phytochemical in Selected Spices/Herb

Parameter	Spices/Her				
	Ginger	Onion		Negro pepper (NP)	Chili pepper (Cp)
Phytochemicals present					
Phenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Quantity of phytochemical present (%) **Phenols**	0.803	0.528	0.627	1.369	1.667
Flavonoids	3.045	2.240	1.157	0.704	0.737
Tannins	0.570	0.609	0.561	0.447	0.460
Alkaloids	0.059	0.128	0.090	0.188	0.677
Saponins	2.952	0.652	2.379	2.295	2.012

Notes: + implies presence of a particular phytochemical in the spice; - implies absence of aparticular phytochemical in the spice/herb

4. 4.2 Functional Properties of Spices/Herbs

The functional properties of the spices analyzed included residual moisture content, pH, water absorption capacity, oil absorption capacity, swelling capacity and solubility index as shown in Table 11.

Negro pepper recorded the highest residual moisture content of 17.75% with prekese recording the least moisture of 6.00%. Chili pepper (7.75%) and ginger (8.50%) were not significantly different ($p>0.05$) from each other whilst NP (17.75%), onion (11.00%) and prekese (6.00%) were different ($p<0.05$) from each other and all other samples.

The mean pH of the spices also showed significant differences ($p<0.05$) between ginger (6.53) and the rest of the spices as it happened in prekese (5.62) and Cp (5.48).
There was no significant difference ($P>0.05$) between NP (5.35) and onoin (5.29). Water absorption capacity was significantly different ($p< 0.05$) among all the spices and ranged from 412.32g/g (onion) to 69.45g/g (prekese). The oil absorption capacity was least in onion ($p< 0.05$) while others had statistically similar values ($p>0.05$).

The swelling properties and solubility index of the spices followed a similar trend as the other functional properties. The swelling properties and the solubility index were highest ($p<0.05$) in onion while prekese had the least swelling properties (3.24%) and NP the least solubility index (12.38%). A significant difference ($p<0.05$) was observed among the spices such as onion (61.41%), Cp (33.33%), ginger (18.22%) and NP (12.38 %). No significant differences ($p>0.05$) existed between Cp (33.33%) and prekse (31.05%).

Table 11: Functional Analysis of Spices/Herbs

	SPICES/HERB

Functional Properties	Negro Pepper **(NP)**	Chili Pepper (**Cp**)	Prekese	Onion	Ginger	**P-Value (P = 0.05)**
Residual Moisture (%)	17.75^{a}	7.75^{c}	6.00^{d}	11.00^{b}	8.50^{c}	< 0.001
pH	5.35^{d}	5.48^{c}	5.62^{b}	5.29^{d}	6.53^{a}	0.015
Water Absorption Capacity(WAC) (g/g)	108.47^{b}	163.33^{b}	69.45^{b}	412.32^{a}	197.25^{b}	< 0.001
Oil Absorption Capacity (OAC) (g/g)	103.29^{a}	80.12^{a}	83.44^{a}	28.92^{b}	93.75^{a}	< 0.001
Swelling property (%)	4.02^{d}	4.55^{c}	3.24^{e}	5.58^{a}	4.85^{b}	0.021
Solubility index (%)	12.38^{e}	33.33^{b}	31.05^{c}	61.41^{a}	18.22^{d}	< 0.001

Note: ^{abcde} means with different superscripts along the same row are significantly different ($p< 0.05$).

Study 2

4.5.0 Evaluation of Frankfurter Sausages treated with Essential Oils from Different Spices/Herbs

4.5.1 Cooking Yield/Loss

The cooking yield of the products as shown in Table 12, showed a range value of 93.41 to 83.93%. The control (86.91%) was statistically similar to those of NPE, CpE and PRE treated samples with values of 93.25%, 93.41% and 89.33% respectively. GE and OE treated samples recorded similar values ($p>0.05$) of 83.93% and 85.98% respectively.

The cooking loss was highest in GE (16.07%) and least in CpE (6.59%) treated products. Ginger essential oil treated- product (16.07%) was not significantly ($p>0.05$) different from OE (14.02%), control (13.09%) and PRE (10.67 %). In like manner, CpE (6.59%), NPE (6.75%) and (10.67) were not significantly different.

Table 12: Cooking Analysis and Proximate Composition of the Products Products P-Values

METER (%)	CONTROL	NPE	CpE	OE	PREZ	GE	**(P = 0.05)**
Cooking Analysis							
Cooking Yield	86.91^{ab}	93.25^{a}	93.41^{a}	85.98^{b}	89.33^{ab}	83.93^{b}	0.110
Cooking Loss	13.09^{a}	6.75^{b}	6.59^{b}	14.02^{a}	10.67^{ab}	16.07^{a}	0.110
Proximate Composition							
Protein	22.84^{ab}	22.89^{ab}	21.54^{b}	23.24^{ab}	23.71^{a}	23.10^{ab}	0.137
Moisture	61.07^{bc}	59.73^{cd}	61.53^{a}	60.67^{bc}	58.99^{d}	60.07^{bc}	0.001
Fat	10.03^{e}	10.65^{d}	11.27^{b}	10.71^{d}	11.61^{a}	11.10^{c}	0.000
Fibre	0.69^{cd}	0.96^{a}	0.80^{b}	0.76^{c}	0.91^{ab}	0.58^{d}	0.000
Ash	3.55^{cd}	3.90^{a}	3.75^{b}	3.43^{d}	3.18^{e}	3.56^{c}	0.000
Carbohydrate	1.82^{a}	1.87^{a}	1.11^{c}	1.16^{c}	1.60^{b}	1.59^{b}	0.000

PARA -

Note:* [abcde]*means with different superscripts on the same row are significantly different (p< 0.05).

4.5.2 Proximate Composition of Products

The proximate composition of the products is shown in Table 12. No significant differences were observed among treatments exceptthe prekese - treated product (23.71%) and the chili pepper product (21.54%) in terms of protein content. Moisture level was significantly higher in CpE (61.53%) and lower in the PRE treated product (58.99%). Fat content was generally highest ($p>0.05$) in PRE (11.61%) than the rest of the treatments. Ash content was relatively highest with NPE (3.90%) and was significantly different from the others. The fibre content was significant among all the samples. However, NPE gave the highest value (0.9%) while CpE and PRE gave values that were statistically similar ($p>0.05$) Lower carbohydrate content was observed in all treatments and ranged from 1.59% (GE) to 1.87% (NPE). The carbohydrate content of the extract ranged between 1.87% in NPE to 1.11% in CpE.

With the exception of NPE, all treatments with essential oils had lower carbohydrate ($p<0.05$) compared to the control.

4.5.3 pH of Products

The pH values of the fresh and cooked products after seven 7, 14 and 21 days of storage are summarized in Table 13. The mean pH of the fresh products were slightly acidic such that the ginger treated sample (GE) recorded the highest pH value of 5.95 and the least pH value (5.52) recorded by the onion treated product (OE). The control (without any essential oil) had a pH value of 5.69. After 7days of storage, the mean pH of the cooked products ranged between 6.14 (control) and 6.01(PRE) with significant differences ($p<0.05$) existing among the values for NPE (6.05), CpE (6.08), OE (6.11) and PRE (6.01). After fourteen (14) days of storage, the pH value of control product did not change (6.14) and was not significantly different from GE

(6.15) as was in the case of CpE (6.11) and OE (6.12). However PRE's pH value (6.09) was the least and was significantly different from NPE (6.17) and other pH values recorded. Thus, pH ranged from 6.17 to 6.09 at the fourteenth day. Further increase in pH values of all the treatments was observed on the 21st day of storage. NPE recorded the highest pH value of 6.36 which was not significantly different from CPE (6.35). Also the pH values of control (6.18), OE (6.17) were not significantly different ($p<0.05$) as was in the case of OE (6.17) and GE (6.16). However, GE (6.16) was significantly different (P<0.05) from the others as it happened in the case of PRE (6.27) after 21 days of storage.

Table 13: Keeping Quality of Products PRODUCTS P- Value

PARAMETER	CONTROL	NPE	CpE	OE	PRE	GE	**(P = 0.05)**
pH Fresh	5.69^{d}	5.78^{c}	5.86^{b}	5.52^{f}	5.62^{e}	5.95^{a}	0.000
Day 7 storage	6.14^{a}	6.05^{d}	6.08^{c}	6.11^{b}	6.01^{e}	6.13^{a}	0.000
Day 14 storage	6.14^{b}	6.17^{a}	6.11^{c}	6.12^{c}	6.09^{d}	6.15^{b}	0.000
Day 21 storage	6.18^{c}	6.36^{a}	6.35^{a}	6.17^{cd}	6.27^{b}	6.16^{d}	0.000
FFA at Storage (%)							
Day 7	1.12^{a}	1.13^{a}	0.56^{c}	0.10^{d}	0.70^{b}	1.08^{a}	0.000
Day 14	1.80	1.60	1.27	1.41	1.68	1.27	0.178
Day 21	3.37^{a}	1.68^{b}	3.19^{a}	1.42^{c}	3.35^{a}	1.27^{c}	0.000
DPPH at Storage (%)							
Day 7	69.38^{a}	64.89^{b}	64.23^{b}	64.73^{b}	62.40^{b}	72.55^{a}	0.007
Day 14	67.39^{e}	95.51^{c}	99.67^{a}	88.19^{d}	96.01^{c}	98.17^{b}	0.000
Day 21	62.90^{f}	88.69^{c}	64.39^{e}	97.84^{b}	83.53^{d}	99.67^{a}	0.000

Note: ^{abcdef}***means of different superscripts on the same row are significantly different (p< 0.05).***

pH of pork used 5.35

4.5.4 Free Fatty Acids (FFA) in the Sausages

Generally, all treatments recorded lower free fatty acids (FFAs), throughout the storage period as shown in Table 13. After seven days of storage, the control, NPE and GE treatments recorded 1.12%, 1.13% and 1.08 % FFA respectively which were not significantly different (p>0.05) from each other. On the contrary, PRE - 0.70%, OE - 0.17% and CpE - 0.56% were significantly different from each other at (p<0.05). On day fourteen, the highest %FFA was 1.80 (control) and the least was 1.61 (NPE). However, these values were not significantly different (p>0.05). At the end of the 21days storage period, the control recorded the highest percentage of free fatty acids (3.68). However, this value was not significantly different (p>0.05) from PRE (3.35%) and CpE (3.19%). Similarly, GE recorded the least FFA of 1.27% which was not significantly different ($p>0.05$) from OE (1.42%). NPE recorded 1.68% which was significantly different ($p<0.05$) from the other treatments.

4.5.5 Antioxidant Activity of Essential Oils of Spices/Herbs

The antioxidant activities of the essential oils of Negro pepper (NPE), chili pepper (CpE), onion (OE), prekese (PRE) and ginger (GE) tested in frankfurter sausages, stored from seven (7) days to twenty one (21) days at freezing temperature of -18 °C are shown in Table 13. The trolox standard curve generated for quantification of antioxidative properties of the essential oils is also shown in Fig.6.

Antioxidant activity was observed in all the products including the control. This activity was generally low after seven days of storage. Percentage of inhibition was higher in the GE (72.55%) but was not significantly different ($p>0.05$) from the control (69.38%) after seven (7) days of storage. No significant differences ($P>0.05$) existed among the other treatments. The NPE (95.51%), PRE (96.01%), and CPE (99.67%) showed their highest scavanging activities at day 14 of storage and were significantly different ($p<0.05$) from each other except NPE (95.51%) and PRE (96.01%). At the end of the storage period of three weeks, GE and OE exhibited stronger antioxidant activity and were significantly different ($p<0.05$) from each other. Thus, the rest of the treatments declined in their inhibition of oxidation at week three, especially CpE - treated product and the control.

Quantifying from the trolox standard curve, it could be observed that very little amount

essential oil from onion of concentration 0.67μM could offer 97.84% inhibition while essential oil from ginger of concentration 0.68μM offered 99.67% inhibition in the third week of this study.

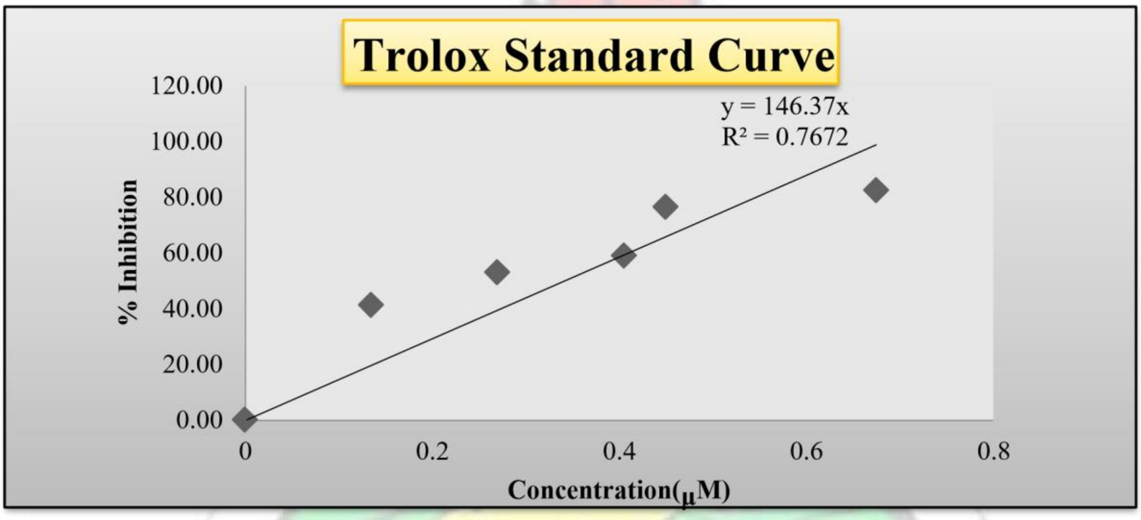


Fig.6: Trolox Standard Curve

4.5.6 Microbial Analysis on the Products

The possibility of microbial inhibition by essential oils of Negro pepper (NPE), chili pepper (CpE), onion (OE), prekese (PRE) and ginger (GE) tested in frankfurter sausages, stored for 21 days at freezing temperature of -18°C are shown in Table 14

The microbial load (TPC x10^{-5}) of the fresh products were 6.46 Log_{10}CFU/g (control),

of the essential oils are needed to offer strong antioxidant property. For instance

6.26Log_{10}CFU/g (NPE), 6.32Log_{10}CFU/g (CpE), 6.20Log_{10}CFU/g (OE), 6.30 Log_{10}CFU/g (PRE) and 6.18Log_{10}CFU/g (GE treated). The products differed significantly ($p<0.05$) acorss all treatments. After 7 days of storage, the cooked products recorded a reduction in the microbial load in all the treatments especially the OE treated and PRE treated which recorded values of less than 25CFU (<5.40 Log_{10}CFU/g). The

TPC x10^{-5} of the products after 14 days storage increased but OE treated and PRE treated values were still less than 25CFU (<5.40 Log_{10}CFU/g). All the others were significantly different ($p<0.05$).

Further increase ($p<0.05$) in the microbial load was observed across all treatments after 21days of storage. OE treated (5.71Log_{10}CFU/g) and PRE treated (5.72 Log_{10}CFU/g) were not significantly different from each other. GE treated (5.66 Log_{10}CFU/g) showed the highest microbial inhibition after three weeks of storage. In general, treatments were safe for consumption after 21 days of storage since the microbial load was below 7.0 Log_{10}CFU/g.

E. coli, Salmonella and Pseudomonas were not present in all the treatments during the 21 days of storage and are reported in the Table 15 as less than one (1) times the dilution factor. There was growth of Staphylococcus in the control and the CpE at all the testing points. There was a gradual increase in growth of Staph in the control as from day 7 to day 21. The CpE treated product however, had higher population on day 7 and declined afterwards. Staph growth in the NPE was observed on Day 7 and Day 21 but not Day 14. In PRE and GE, Staph growth was observed in Day 7 only.

No Staph growth was observed in OE products throughout the storage period.

Table 14: Microbial Analysis of Cooked Sausages treated with the different Essential Oil (Log_{10}CFU/g)

MICROBIAL TYPE	TREATMENT	7- DAYS STORAGE	14- DAYS STORAGE	21- DAYS STORAGE
	CONTROL	6.36^a	6.40^a	6.59^a
	NPE	5.85^c	6.15^c	6.38^b
	CpE	6.28^b	6.30^b	6.34^c
TPC x10^{-5}	OE	$<5.40^e$	$<5.40^e$	5.71^d
	PRE	$<5.40^e$	$<5.40^e$	5.72^d
	GE	5.57^d	5.63^d	5.66^e
P-Value		0.000	0.000	0.000
	CONTROL	<-2	<-2	<-2
	NPE	<-2	<-2	<-2
	CpE	<-2	<-2	<-2
E.COLI	OE	<-2	<-2	<-2

	PRE	<-2	<-2	<-2
	GE	<-2	<-2	<-2
STAPH	CONTROL	5.86	5.95	6.18
	NPE	<5.40	<-5	<5.40
	CpE	5.77	<5.40	<5.40
	OE	<-5	<-5	<-5
	PRE	<5.40	<-5	<-5
	GE	<5.40	<-5	<-5
SALMONELLA/ PSEUDOMONAS	CONTROL	<-5	<-5	<-5
	NPE	<-5	<-5	<-5
	CpE	<-5	<-5	<-5
	OE	<-5	<-5	<-5
	PRE	<-5	<-5	<-5
	GE	<-5	<-5	<-5

Note: [abcde] means with different superscripts along the same column are significantly different (p< 0.05). <-5 and <-2 imply no growth

4.5.7 Sensory Evaluation

The panelists evaluated the products based on the attributes such as colour/appearance, aroma (odour), taste, juiciness, tenderness and overall acceptability. Different levels of the attributes were chosen based on a 9-points hedonic scale from extremely bad (1) to extremely good (9) as shown in Table 15.

The colour of all the products were liked slightly (6) to moderately (7) without any significant differences (p>0.05) among treatments. The panelists also liked the aroma slightly - moderately across all treatments. There was, however a significant differences (p<0.05) among some of the treatments. GE (7.09) had the best aroma (odour) and was significantly different (P<0.05) from OE (6.20), PRE (5.71) and NPE (5.60) - the worse aroma. Significant difference was not shown among the aroma of the control (6.38), CpE (6.82) and GE (7.09).

The tastes of the products were slightly accepted. CpE (6.82) was chosen as the best product in terms of taste followed by GE (6.40), OE (6.16) and the control (5.91), but did not show significant differences (p>0.05) among each other. PRE and NPE showed the least score (5.20), and 5.31 respectively for taste and panelists were indifferent about their taste. No significant differences (p>0.05) existed between the two least scored treatments for taste. Panelists rated the products as slightly juicy - moderately juicy with OE being moderately juicy (7.02) and the rest slightly juicy. The result also indicates that GE (6.36) was not significantly different (p>0.05) from OE (7.02) in terms of juiciness. They were however, significantly different from the rest of the products at confidence level of 95%.

The products were generally described as slightly tender. CpE (6.73) was better in term of tenderness than the other products but was not significantly different (p>0.05) from OE (6.56) and GE (6.47). Control (5.89), NPE (5.82), OE (6.56), GE (6.47) and PRE (5.93) were not significantly different (p>0.05) from each other. In overall acceptability, CpE (7.20) was chosen as the best over the other treatments but was not significantly different from the GE (6.80). The control (6.16), OE (6.07), were not significantly different (p>0.05) from each other. The NPE (5.02) and PRE (5.44) were the least accepted, probably based on the taste and aroma according the general comments from the respondents.

Table 15: Sensory Evaluation of Products

	PRODUCTS						
ATTRIBUTE	**CONTROL**	**NPE**	**CpE**	**OE**	**PRE**	**GE**	**P-Value (P = 0.05)**
COLOUR	7.04	6.44	7.09	6.58	6.82	6.78	0.394
AROMA (ODOUR)	6.38^{abc}	5.60^{c}	6.82^{ab}	6.20^{bc}	5.71^{c}	7.09^{a}	0.000
TASTE	5.91^{ab}	5.31^{b}	6.82^{a}	6.16^{ab}	5.20^{b}	6.40^{a}	0.004
JUICINESS	5.76^{b}	5.87^{b}	6.04^{b}	7.02^{a}	6.04^{b}	6.36^{ab}	0.041
TENDERNESS	5.89^{b}	5.82^{b}	6.73^{a}	6.56^{ab}	5.93^{ab}	6.47^{ab}	0.060
OVER ALL ACCEPTABILITY	6.16^{b}	5.02^{d}	7.20^{a}	6.07^{bc}	5.44^{cd}	6.80^{ab}	0.000

Note: $^{abcd\ e}$***means with different superscripts along the same row are significantly different (p< 0.05).***

4.6 Cost of Producing the Product

The cost of producing a kg of the products treated with the essential oils as compared to the control is shown in Table 16. From the table, the cost of producing a kg of the control, NPE, CpE, OE, PRE and GE products are GH¢15.00, GH¢23.00, GH¢24.00, GH¢23.00, GH¢23.50, and GH¢24.50 respectively. With respect to the cost of control product, 53.00% additional cost was incured to produce NPE and OE treated products, 56.67% more was spent on producing PRE treated product, 60.00% more for CpE and 63.33% more Ghana Cedis was spent for the production of GE treated product. In all, it was more expensive producing a kg of the ginger oil treated product (GE) than the rest.

Table 16: Cost Analysis of the Sausage

Product	Cost of spice/herb (GH¢)	Cost of oil extration used(GH¢)	Cost of essential production (GH¢)	Total cost of due to use of (GH¢)	Increase in cost product/kg extract GH¢
Control	15.00	-	-	**15.00**	-
NPE	15.00	5.00	3.00	**23.00**	**8.00 =** 53.00%
CpE	15.00	6.00	3.00	**24.00**	**9.00 =** 60.00%
OE	15.00	5.00	3.00	**23.00**	**8.00 =** 53.00%
PRE	15.00	5.50	3.00	**23.50**	**8.50 =** 56.67%
GE	15.00	6.50	3.00	**24.50**	**9.50 =** 63.33%

Note: *Increase cost = total cost - [cost of spice used+cost of oil extraction (0.5g)] % increase in cost = increase in cost/ cost of kg of product x 100.*

CHAPTER FIVE

5.0 DISCUSSION

Study 1 Phase 1

5.1 Assessment of Spices/Herbs Used in Kumasi Metropoolis

5.1.1 Demographic Characteristics of Respondents (Consumers/Domestic Users) The consumers/domestic users of spices/herbs in meat/food products in the Kumasi were mostly females and could be attributed to fact that women are predominantly responsible for the preparation of meat and other dishes for the dining tables in our homes. This agrees with the findings of Aniedu *et al*. (2007) who observed women as major stakeholders in the provision of food to the dining table. The age bracket of these respondents agrees with report from Medium Term Development Plan for Kumasi Metropolitant Assembly (MTDPKMA), (2010-2013), which states that population of Kumasi metropolis is dominated by the youth. The respondents were mostly Akans and could be attributed to the fact that the metropolis is the traditional capital of the Ashanti Kingdom. Akan is a multi-dialect tribe consisting of Ashanti, Fante, Akuapem, Assin, Ahanta, Akyem and other ethnic groups and forms the major tribe in the metropolis and the country at large. The numerous tribes (19) recorded could be explained to mean that Kumasi is a commercial hub of Ghana due to the existence of the largest open – air market (market existing outside rather than inside a building) in West Africa (the Central Market) (MTDPKMA, 2010-2013), which attract people from all walks of life making it cosmopolitan in nature.

The religious background checks showed that majority of the respondents were Christians. This could be due to the fact that Christianity is the predominant religion among the Akans in the country. This observation is similar to that of MTDPKMA (2010-2013), which reported on the profound dominance of Christians in the Metropolis. Most of the respondents were married probably because most were engaged in business/trade activities that make them financially sound to be responsible for the home. As the capital of the second most urbanized region in the country after the Greater Accra Region, family planning was evident as majority of the respondents had a household size of less than four (4). Reasonably, small household size could also be linked to the fact that most of the respondents were in the active population bracket. According to MTDPKMA (2010-2013), the age dependency ratio for the metropolis is 1: 0.7, implying that the economically active population has less people depending on them.

The metropolis has several public and private educational institutions from basic to secondary to tertiary which the residents can take advantage of. It is therefore much expected that most of the respondents were educated with just few of them having no formal education. The good percentages of respondents with secondary and tertiary education respectively are an indication that the modern city dweller attaches importance to formal education even to the highest level. In respect of occupation, most of the respondents (56%) engaged in business activities and few as civil servants. This could be explained that most people are attracted to the metropolis due to the fact that it is a commercial hub of the country with the formal private and informal private sectors engaging the majority. According to MTDPKMA (20102013), the occupational distribution of the city has 22.70 and 62.20% formal private and informal private sectors respectively with the public sector taking only 8.40%.

5.1.2 Spices/Herbs and their Preferred Forms Used in Meat/Food by Consumers/Domestic Users in Kumasi Metropolis

The frequency of use of internationally recognized spices/herbs showed that that ginger, onion, chili pepper and garlic, were the most commonly used spices/herbs in the metropolis. This is in agreement with Sherman and Billing (1999), who reported that ginger, onion, chilies are the most frequently used spices in India and Ghana for meat-based recipes. These authors reported also that ginger and onion are the most frequently used spices in Southwest China, onion and ginger in Northeast China, whilst onion and chilies are most frequently used spices in Southern U.S.A and Nigeria. They also reported on the frequent of use of garlic in several countries where their research covered e.g. Thailand, Philippines and Malaysia. Negro pepper and prekese were the indigenous Ghanaian spices patronized most by the respondents. This could be attributed to the fact that these spices give special flavour to soup and other meat based recipes in which they are utilized; and a favorite to most of indigenes of the city.

Though very few respondents expressed concern about the use of spices in the whole form, most of them preferred to obtain spices/herbs in powder or whole form because they claim it is easier to use the powder form but also commented that the whole form is less or possible not adulterated.

5.1.3 Common Uses and Misgivings of Spices/Herbs in Meat/Food Products by Consumers/Domestic Users in Kumasi Metropolis

Spices/herbs are mostly utilized in stew, soup, sausages, kebabs or jollof rice probably because these are the common meat based recipes patronized by the respondents in the metropolis. Usually appetite is aroused with respect to good flavour of recipes especially when such recipes are 'injected' with appropriate spices/herbs.

The few respondents who claim spice/herb use could cause ill-health was also evident in respondents' misgivings on the use of spices/herbs where respondents repeated their claim that spice/herb use could cause health problems but did not specify the kind of health problem. From the misgivings stated by the respondents it is clear that spices/herbs users in the metropolis do not think spices/herbs are expensive probably because they spend just about 2-10% of their monthly income of GH¢100.00 to GH¢500.00 and thus, do not affect them economically. Those who claim that the use of spices/herbs alters food taste need education on the use of spices (usually very small quantities).

5.2 Processors/Vendors of Meat Response

5.2.1 The Demographic Characteristics of Processors/Vendors of Meat Products in Kumasi Metropolis

The higher number of males recorded for the processors/vendors of meat products than in the consumer/domestic users' response could be attributed to the fact that more males are involved in processing of meat products than females due to the complex processes associated with meat processing. The higher response from the females in this study could also be explained from the fact that more vendors of meat - based recipes or meat products were sampled and that most of them were females.

The percentage of people from other tribes (apart from Akan) as processors/vendors of meat products was higher done what was recorded for consumers/domestic users of spices/herbs. This is an indication that most of these tribes especially Frafra, Hausa, Dagaati, Sisaala etc., who hail from the Northern part of the country are normally engaged in meat products sales. Although there were more Christians than Muslims, the number of Muslims in the processor/vendor category was higher than that of the consumer/domestic users probably due to the fact that Muslims would want to eat meat - based recipe or product from animals slaughtered in the halal way. More Muslims were into processing and selling of meat products to ensure their colleaques in the same faith get what they want and keep to the doctrines of their faith without a compromise.

The household size of more than four for majority of the respondents implies that respondents were not over burdened due to large family size. Most of the respondents had secondary level education probably because people secure capital after secondary education and start business (petty trade) which dominates commercial activities in the metropolis.

5.2.2 Preferred Forms and Common Uses of Frequently used Spices/Herbs by Processors/Vendor in Kumasi Metropolis

The response from processors/vendors showed that ginger, onion and chilies were frequently utilized in meat products as compared to the response from the consumer/domestic users. This agrees with report from Sherman and Billing (1999), as discussed under consumer/domestic users' response above. Majority of the processors/vendors preferred powdered spices/herbs to other forms though they made use of other forms. Their preference could be attributed to the fact that the powdered form is easily worked into the meat products (Adeleke and Odedeji, 2010). Respondents mostly use these spices in making kebabs and sausages probably because these two products are readily accepted by consumers/domestic users in the metropolis than other meat products. This could imply that when processors/vendors serve custormers with these products they will make more sales.

5.2.3 Misgivings on the Use of Spices/Herbs by Processor/Vendors of Meat Products in Kumasi Metroplis

The study also showed that majority of the respondents did not think spices/herbs usage pose any health concern. The few who claimed spice/herbs usage could pose health problems stated that such health problems could be heart diseases, allergy and stomach clumps. Invariably, some individuals may be allergic to some of these spices or may experience stomach upset after taken food items prepared with some spices/herbs. This could be illnesses caused by ingesting spices that are themselves contaminated with bacteria, fungi, or animal faeces (Sherman and Billing, 1999). It could also be caused by ingesting excess plant secondary compounds and essential oils (Sherman and Billing, 1999).

Besides, the phytochemicals in many spices/herbs have mutagenic, teratogenic, carcinogenic, or allergenic properties according to Ames *et al*. (1990) and Beier and Nigg (1994). Health complaint from respondents could be legitimate since for instance, chili pepper (in small quantities) has antimicrobial and therapeutic effects; however, ingestion of large amounts of capsaicin (found in chili) has been associated with necrosis, ulceration, and carcinogenesis (Surh and Lee, 1996). In effect, too much of a good thing can be bad.

Misgivings reported in this study on the use of spices/herbs such as change of taste of food etc. can be compared to that reported by Govindarajan (1985), which state that, spices/herbs could disguise the smell and taste of spoiled foods. Thus, people could conceal foul smell and bad taste of spoiled meat-based recipe with more pungent spices/herbs and sell to innocent consumers/domestic users.

Study 1 Phase 2

5.3 Phytochemical Content of Spices/Herbs Used

The phytochemical screening of the spices/herbs showed that phenols were highly present in chili pepper, Negro pepper, ginger, preksse and least in onion. The presence of phenols in chili pepper, Negro pepper, ginger and prekese is supported by EmanuaelIkpeme *et al.* (2014), Osabor *et al.* (2015), Belewu *et al.* (2009) and Uyoh *et al*. (2013) respectively. According to Singh and Sawhney (1988), phenols are toxic to the growth and development of pathogens. They can therefore be considered to have antimicrobial and antifungal effect hence their use in disinfectants.

Flavonoids were high in ginger, onion and prekese whereas Negro pepper and chili pepper recorded the lowest. The presence of flavonoids in the above named spices/herbs is comparable to what was reported by Bhargava *et al*. (2012), Abuga

(2014), Uyoh *et al.* (2013), Osabor *et al.* (2015) and Sun *et al.* (2007). According to Kar (2007), flavonoids are aromatic compounds used as antioxidants or free radical scavengers. In effect, ginger, onion and prekse extracts are expected to offer more antioxidant activity than the other spices.

There were more tannins in onion, ginger and prekese than Negro pepper and chili pepper. Abuga (2014), Bhargava *et al*. (2012), Belewu *et al.* (2009), Uyoh *et al.* (2013) and Emanuael-Ikpeme *et al.* (2014) also reported similar findings in their studies. According Belewu *et al.* (2009) tannins have oxidation inhibiting activity and are assumed to result from the presence of gallic and diagollic acids.

The presence of alkaloids in the spices/herbs used in this study is comparable to what was reported by Sun *et al.* (2007), Abuga (2014), Belewu *et al.* (2009), Osabor *et al.* (2015) and Uyoh *et al.* (2013). Research conducted by Molyneux *et al.* (1996), shows that alkaloids function in defense of plants against herbivores and pathogens (microorganisms) and thus acts as antibacterial and antifungal compounds.

The study also showed that the spices/herbs used contain saponins. This observation is similar to the work reported by Bhargava *et al*. (2012), Ugwoke and Ezugwe

(2010), Emanuael-Ikpeme *et al.* (2014), Osabor *et al.* (2015) and Uyoh *et al.* (2013). Okwu (2003) reported that saponin is used to prepare insecticides, steroid hormones and other forms of drugs.

The functions of the various phytochemicals discussed indicate that the spices/herbs used in this study could offer some level of antimicrobial and antioxidant activities to the sausages prepared. The differences in the phytochemical content of spices reported by different researchers from different locations could be attributed to differences in the agro-climatic conditions, maturity and genotype of the spices (Zechariah, 2008) as well as the reagents and apparatus used.

Study 2

5.4 The Physicochemical Properties of Products

The pH of the spices and the pork used were all acidic making the final sausage formed also acidic. This offered some level of protection to the products since higher pH promotes microbial growth in sausages and products spoilage (Cross and Overby, 1988 and FAO, 2007). The pH of the pork used is also indicative that the animals used were not overstressed prior to slaughtering. According to FAO (2007), stressing of animals prior to slaughtering causes exhaustion and depletion of glycogen reserves and reduces post-mortem glycolysis while lactic acid production increase resulting in lowering of pH towards acidity but on the contrary, when the pH is high after slauthering, such meat holds more water and spoils quickly due to high pH and dry surface which encourage bacterial growth.

There was an increase of 0.21(GE) to 0.65(OE and PRE) points in the pH of the products after cooking and storage for three weeks. Similar trend was reported by Akwetey *et al.* (2012) where the products had a cumulative increase of 0.38 points. This pH increase could be attributed to addition of alkaline phosphate to the products (Puolanne *et al*., 2001 and Akwetey *et al*., 2012). Furthermore, the pH values are in agreement with Al -Assaf and Abdullah (2005), who stored frankfurter sausages treated with natural anti-oxidants from garlic, coriander and paprika to check lipid oxidation. At the end of storage period of 55 days at 4°C, the pH were in a range of 6.47- 6.53.

Higher cooking loss was recoreded for products with less pH (GE, OE and the control) and vice versa for products with higher pH values after 21days of storage could probably be due to the fact that higher pH encourages water holding capacity which in turn improves cooking yield. This agrees with report by Puolanne *et al*. (2001) which that the use of pH- raising phosphate improves water holding capacity of cooked sausages. It could also be argued that the reduction in cooking loss of CpE and NPE products is a combined effect of essential oils from the spices and the phosphate used.

The higher levels of protein found in the products is comparable to Akwetey and Yamoah (2013) who reported that protein content for control pork patties was 24.57% when they produced low fat patties with solar dried plantain flour. On the contrary, Gaschler (2012) reported lower protein content of 10.62% - 10.13% when she combined pork, turkey and beef in smoked sausages stored for 90 days. The higher protein content in the current study could be attributed to the higher percentage of pork used in the formulation with no extenders.

Low fat content recorded could be attributed to low level of fat added in manufacture as well as lean meat used. However, it could be seen that the essential oil added up unto the fat content causing a little rise in fat of products treated with essential oils, with prekese oil treated products recording a higher increase than other treatments. The low fat recorded is in agreement with Mendez-Zamora *et al.* (2015), who recorded % fat of

8.90 - 13.35 when they incorporated 13. 65 -16.55% fat to inulin and pectin treated sausages (41.93% pork and 10.98% beef).

In the same study, the authors reported high moisture level of 58.31% - 61.14% upon adding 20.37% ice to the products. The moisture content in this study was in consonance with earlier report of Mendez-Zamora *et al.* (2015). The value of 58.99 - 61.53% was obtained when 18.25% ice cube was added to the formulation in this study. It could be deduced that a good quantity of moisture was bound to the product and did not suffer much purge leading to the high cooking yield observed across all treatments.

Ash content reported in this study was however lower than what was observed by Mendez-Zamora *et al.* (2015). The differences could be attributed to the inulin and pectin as well as other ingredients used in Mendez-Zamora's study.

The carbohydrate contents recorded in this study were quite lower and could be due to the fact that no extenders and binders were added to the formulation, contrary to Huda *et al*. (2010) who recorded carbohydrate content of 6.69- 21-59% when samples of chicken sausages marketed in Malaysia were analyzed for proximate composition. The increase in carbohydrate content in sausages could be due to the increase of starch content which substituted for raw meat in the production of the sausages. Manufacturers usually add starch to reduce processing cost and increase profit margin.

5.5 Keeping Quality of Products

5.5.1 Free Fatty Acids (FFA) Production

The FFA content in a product is an indication of the quality of the product (Clucks and Ward, 1996). In this study, the GE and OE had the highest ($p<0.05$) reduction in FFA production and in effect offered the best protection at the end of the 21 days storage. This is in line with the study conducted by Idris *et al.* (2010) who reported that various concentration of ginger inhibited FFA production in smoked fish stored for eight (8) weeks.

5.5.2 Antioxidant Activity of Products

Some level of antioxidant activity observed in the control could probably be attributed to the spice mix added to the sausages during preparation. The mixture included garlic, pepper, onion and nutmeg. Decline in antioxidant activity may result from the fact that the mixture was not concentrated enough to offer long term antioxidant effect on the products. Thus, spices added to meat preparations offer some level of protection against lipid oxidation. This is in agreement with Virendra *et al*. (2013) who reported that spices like ginger, garlic and turmeric have antioxidant activity. Rehman *et al*. (2003) also commented that the antioxdant activity of ginger is comparable to that of the synthetic antioxidants, BHA and BHT. In addition to this,

Kikuzaki and Nakatani (2006) reported that 2 of the 5 gingerol-related compounds and 8 diarylheptanoids isolated from ginger rhizomes exhibit higher antioxidative activity than αtocopherol.

The general low antioxidative activity exhibited after one week storage could be as a result of less or minimal lipid oxidation of the products, and that the antioxidative potential of the essential oils had not been fully put to test. A higher inhibition was shown at week two (14 days) of storage probably because oxidation had increased and caused the oils to scavenge as many free radical as they could.

Onion and ginger essential oils were at their best in the third week demonstrating the ability to prevent lipid oxidation in emulsion type sausages for longer storage periods.

The antioxidant activity shown by the essential oils in this study is in agreement with Karioti *et al*. (2004), who evaluated the antioxidant ability of the essential oils of *Xylopia aethiopica* leaves, stem bark, root bark and fresh and dried fruits from Ghana using different methods. The essential oil of fresh fruits however, possessed the highest capacity for reducing DPPH, whereas the oil isolated from leaves showed the highest ability to scavenge superoxide anion radical. According to the authors, the main compounds that constituted the oils included different levels of germacrene - D, α- and β-pinenes, and trans-m-mentha-1(7), 8-diene.

The results also agree with Sacchetti *et al*. (2005) who by the DPPH method, found out that *Rosmarinus officinalis* and *Cymbopogon citratus* oils showed major effectiveness as antioxidant, with radical inhibition in the range 60–64%, lower than that of the reference, *Thymus vulgaris* oil (76%). Using the same method as stated above, Chizzola *et al*. (2008) reported that the essential oils of *T. vulgaris* which is rich in thymol presented antioxidant activity. In addition, Jukić and Miloš (2005) demonstrated, by DPPH and FRAP (Ferric ion reducing antioxidant power) methods, that the phenolic chemotype *T. vulgaris L.* oils, possessed stronger antioxidant properties than the nonphenolic phenotype. Sun *et al*. (2007) also reported that increased amounts of carotenoids, phenolics, flavonoids and ascorbic acid in the fully ripe fruits of chili pepper exhibit higher antioxidant activity. Once again Soawaluck and Paisooksantivatana (2009) reported that the essential oil from rhizome of *Zingiber montanum* from the northern and eastern parts of Thailand is effective as natural antioxidant to be used in medicinal and food products to promote human health and prevent diseases. They indicated this when their study recorded 57.63 to 80.80% inhibition of oxidative rancidity as compared to 62.90 - 99.67% inhibition in this study.

5.5.3 Microbial Load of Products

The microbial load of the fresh product (control) was high and this indicates a high level of contamination from the Kumasi Abattoir Company Ltd. A similar observation was made by Sodzim, (2012) who reported initial microbial load of 6.1log$_{10}$CFU/g on fresh pork bought from the same source. According to the author, the value increased to 7.03log$_{10}$CFU/g, a day after storage at room temperature, a figure above the maximum recommended limit of 7 log$_{10}$ CFU/g set by ICMSF (1986) for Aerobic Plate Count in processed meat. Besides, a similar observation was made by Zakpaa *et al.* (2009) on meat products obtained from some markets within the Kumasi Metropolis.

Comparing the essential oil treated products to the control, it is obvious that the volatile oils offered some level of microbial inhibition to the products even before cooking. After a day's storage, cooked sausages had their microbial load reduced across all treatments. This significant result could be attributed to the smoking and cooking of the products before storage. Literature has reported severally on the ability of smoking and cooking to offer protection to products by killing and inhibiting microbial growth. For instance, the Northern Europeans, according to Young and White (2008), keep their fresh sausages by a process of smoking to help preserve the meat during warmer months. USDA (1999) also stated in its Distance Learning Course Manual for Safe Practices for Sausage Production that smoking imparts chemical substances such as acids, carbonyls, phenolics and polycyclic hydrocarbons on the products which in effect inhibit bacterial growth in the finished products. The Department also affirms that cooking inhibits the spoilage bacteria in meat products.

The reduction in the microbial load of the products after cooking could also be attributed to the addition of the essential oils; owing to the fact that more reduction was seen in the essential oil treated products than the control product. This is in line with Lobisco (2015), who reported that essential oils (EOs) are excellent antimicrobial agents sometimes used in food packaging. The author found that some clinical isolates were susceptible to oregano and cinnamon essential oils. The general microbial load increase in the sausage products is in line with report by Rahman (1999b), who found that during freezing, about 60% of the viable microbial population dies but the remaining population gradually increases during frozen storage. The absence of *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* in the product is probably an indication of good hygienic processing conditions that was observed during the product manufacture, preventing further contamination of the meat products.

However, the presence of Staph could result from slaughtering contaminations at the abattoir. The reduction in the Staph population of essential oil treated products is an indication of antimicrobial activities of the oils and thus in agreement with Tassou *et al*. (1996) and Skandamis & Nychas (2000), who reported that essential oils are capable of inhibiting microbes. The antimicrobial action of essential oils may be due to impairment of a variety of enzyme systems including those involved in energy production and structural component synthesis (Conner *et al*. 1984).

5.6 Sensory Evaluation of Prooducts

Products of all treatments did not show any significant differences in the appearance/colour probably due to the minute amount used which could not influence colour development. The aroma of products was significantly affected, and could be attributed to the concentrated nature of the essential oils. Ginger and onion were best accepted for their aroma and this could stem from the fact that consumers are used to these odours and are widely accepted in food items (Sherman and Billing, 1999). Products from ginger and onion also had less microbial contaminations and oxidative rancidity and therefore offered less or no interference with the odour of these products.

The rejection of the aroma and taste of prekese and Negro pepper treated - products could be attributed to their unusual use in sausage production. The attributes of aroma and taste (flavour) was probably the basis of overall acceptability of the products.
This is in line with a study done by Dransfield *et al.* (1984) who found that Irish,

English, French and Belgian panels tended to value flavour more highly than tenderness and juiciness. Thus the panelists in this study were in line with the English, F rench, Belgian and the Irish in terms of sausage palatability. The overall acceptability of the essential oil treated - products is comparable to report by Jedlicka (2009) who produced meat with acceptable eating qualities after the inclusion of essential oils in the diets of steer calves.

5.7 Cost of Producing the Products

The cost of producing frankfurter sausages treated with essentials oils was higher than the control due to the cost of purchasing the spices and extracting the oil from the spices.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

- Spices/herbs commonly used by the respodents in the metroplis are

  ○ Ginger ○ Onion

  ○ Chili Pepper ○ Prekese ○ Negro Pepper

- These spices/herbs contain phytochemicals such as ○ Phenols, ○ Flavonoids, ○ Tannins, ○ Saponins ○ Alkaloids ○ Essential oils

- Utilization of essential oils from the spices/herbs in frankfurter sausages indicated that the oils could:

  ○ Reduce the produ ction of Free Fatty Acids (FFA), ○ Be used as an antioxidant agent to inhibit lipid oxidation ○ Be used as anti microbial agent especially to control *Staphlococcus aureus*

  ○ Be used without adversely affecting the organoleptic qualities of the the products.

6.2 Recommendations

Further research should investigate the chemical composition of the essential oils and how these chemicals affect their functions. The study could also investigate the effect of storage of these essential oils to ascertain their max imum utilization as antioxidant

From the survey, it could be concluded that and antimicrobial agent in the sausage industry. Besides, the length of storage of products could be extended further to check

how far the test ingredients could go.

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APPENDICES

APPENDIX 1

ANALYSIS OF VARIANCE (ANOVA) FOR VARIOUS PARAMETERS

Residual Moisture (RM)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	252.5250	63.1313	93.53	<.001
Residual	10	6.7500	0.6750		
Total	14	259.2750			

Oil Absorption Capacity (OAC)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	10906.425	2726.606	2129.83	<.001
Residual	10	12.802	1.280		
Total	14	10919.227			

Solubility Index (SI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	4318.894	1079.724	290.69	<.001
Residual	10	37.144	3.714		
Total	14	4356.038			

Swelling Power (SP)

Source of variation d.f. s.s. m.s. v.r. F pr. Trt 4 9.2876 2.3219 4.72 0.021

Residual	10	4.9188	0.4919
Total	14	14.2064	

Water Absorption Capacity (WAC)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	4	214045.979	53511.495	16259.03	<.001

3.291

214078.891

Residual
10
32.912
Total

14

pH of Spices

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
	4	3.3153	0.8288	5.26	0.015
Residual	10	1.5755	0.1575		
Total	14	4.8908			

Cooking Yield and Loss

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
COOKING	Between Groups	229.611	5	45.922	4.878	.011
	Within Groups	112.969	12	9.414		
	Total	342.581	17			
COOKING YIELD	Between Groups	229.611	5	45.922	4.878	.011
	Within Groups	112.969	12	9.414		
	Total	342.581	17			

Product Proximate Composition

	Source of variance	Ss	df	ms	F	Sig.
PROTEIN	Between Groups	7.992	5	1.598	2.088	.137
	Within Groups	9.188	12	.766		
	Total	17.179	17			
MOISTURE	Between Groups	12.833	5	2.567	8.646	.001
				.297		

Trt

LOSS

		Within Groups	3.562	12				
		Total	16.396	17				
	FAT	Between Groups	4.613	5	.923	109.538	.000	
					.008			
		Within Groups	.101	12				
		Total	4.714	17				
Within 12	FIBRE	Between Groups	.294	5	.059	11.737	.000	Groups .005 .060
		Total	.355	17				

		s.s	df	m.s	F	Sig.
ASH				.188		
	Within Groups	.055	12	.005		
	Total	.994	17			
CARBO - HYDRATE	Between Groups	1.569	5	.314	310.558	.000
	Within Groups	.011	11	.001		
	Total	1.580	16			

pH of products (sausages)

	Source of Variation	s.s	df	m.s	F	Sig.
FRESH	Between Groups	.374	5	.075	1.347E3	.000
	Within Groups	.001	12	.000		
	Total	.375	17			
DAY 7	Between Groups	.036	5	.007	108.683	.000
	Within Groups	.001	12	.000		
	Total	.037	17			
DAY 14	Between Groups	.014	5	.003	41.683	.000
	Within Groups	.001	12	.000		
	Total	.015	17			
DAY 21			5	.023	698.700	.000
	Between Groups	.939	5	.000	40.985	.000

	Between Groups	.116				
	Within Groups	.000	12			
	Total	.117	17			

FFA of Products

	Source of Variation	s.s	df	m.s	F	Sig.
DAY 7	Between Groups	1.513	5	.303	276.433	.000
	Within Groups	.007	6	.001		
	Total	1.519	11			

DAY 14	Between Groups	.507		.101	2.233	.178
	Within Groups	.273		.045		
	Total	.780	11			
DAY 21	Between Groups	10.427	5	2.085	275.004	.000
	Within Groups	.045	6	.008		
	Total	10.472	11			

Antioxidant Activity of Essential Oil in the Products

	Source of Variation	s.s	df
DAY 7	Between Groups	145.037	5
	Within Groups	17.189	6
	Total	162.226	11
DAY 14	Between Groups	1474.642	5
	Within Groups	1.276	6
	Total	1475.917	11
DAY 21	Between Groups	2561.806	5
	Within Groups	1.493	6
		2563.299	
			5
			6

FRESH	Between Groups	.153	5	Total	11
	Within Groups	.000	12		
	Total	.153	17		

Microbial Load (Total Plate Count- (TPC x10^{-5}))

	Source of Variation	s.s	df

	Between Groups	2.756	
	Within Groups	.000	12
	Total	2.757	17
DAY 14	Between Groups	3.129	5
	Within Groups	.000	12
	Total	3.129	17
DAY 21	Between Groups	2.579	5
	Within Groups	.000	12
	Total	2.579	17

m.s	F	Sig.
29.007	10.125	.007
2.865		
294.928	1.387E3	.000
.213		

DAY 7	5

512.361	2.059E3	.000
.249		

m.s		
.031	916.800	
.000		
.551	1.654E4	.000
.000		
.626	1.877E4	.000
.000		
.516	1.547E4	.000
.000		

Sensory Characteristics of Sausages

Source of variance

	s.s	df	m.s	F
COLOUR/ APPEARANCE			Between Groups	
		5	2.877	1.040
		264	2.765	
		269		

F	Sig.
	.000

			Sig.
			14.385
			.394
5	15.731	4.625	.000

138

264	3.402
269	

5

17.784 3.494 .004

264 5.090

269

5 9.497 2.359 .041

264 4.025

269

5 7.073 2.149 .060

264 3.291

269

5 29.648 7.740 .000

Within Groups 1011.200

Total 1159.441

264 3.830

269

Within Groups 744.385

Total

AROMA Between Groups 730.000

Within Groups TASTE

78.656

Total

898.044 976.700

Between Groups 1343.778

1432.700

Within Groups Total

JUICINESS

88.922

Between Groups 1062.622

1110.107

Within Groups

Total

TENDERNESS Between Groups

47.485

35.367

APPENDIX 2a

Within Groups 868.933

Total 904.300

OVER ALL ACCEPTABILITY Between Groups 148.241

<u>QUESTIONNAIRES</u>

Kwame Nkrumah University of Science and Technology

Department of Animal Science

<u>Consumers/ Domestic Users Questionnaire</u>

On

THE USE OF DIFFERENT SPICES IN KUMASI METROPOLIS

This questionnaire seeks to select responses from domestic users/consumers on the use of spices in meat products. Kindly assist in providing accurate information on the questions raised by ticking the appropriate answers.

SECTION A: PERSONAL DATA date ……………………

1. Gender: (a) Male (b) Female
2. Age: (a) ≤25 b) 26-35 (c) 36-45 (d) 46-50 (e) above 50

3. Location ………………………………………………………………………………

4. Tribe: ……………………………………………………………………………….

5. Religion: (a) Christianity b (b) Islam (c) Others

6. Marital Status: (a) Single (b) Married (c) Divorced (d)

Widowed

7. Household Size: (a) less than 4 (b) 4-6 (c) above 6

8. Occupation: (a) Civil Servant (b) Business / Trader

(d) Others specify ……………………………………………………………

9. Level of Education: (a) Basic (b) Secondary (c) Tertiary (d) No formal **SECTION B**

1. Are you familiar with spices and their use?

a) Yes b) No

2. If yes, which spices or herbs are you familiar with?

a) Cinnamon g) Negro Pepper (*Hwentea*) m) Aniseed

b) Chilies h) Mace n) Cumin

c)Coriander i) Cardamom o) Ginger

d) *Prekese* j) West African Black Pepper p) Calabash Nutmeg (*Awer Amba*)

e) Cloves j) African Locust Bean (*Dawadawa*)

f) Nutmeg l) Grains of Paradise (*Soro Wisa*)

q) State others: ……………………………………………………………

3. Do you use spices in your food preparations?

a) Yes b) No

4. If yes, which of the spices mentioned in ques.2 above do you commonly use?

………………………………………………………………………………

………………………………………………………………………………

5. Which kinds of foods do you use this/these spices?

a) Soup b) stew c) jollof rice

d) State others:

………………………………………………………………………………

…………………………………………………………………………………

6. Are you familiar with meat products?

   a) Yes b) No

7. If yes, which meat products are you familiar with?

   a) Sausages b) Burgers c) Meat loaf d) Barbecue e) Khebab

   f) State others:……………………………………………………………

8. Which of the meat products mentioned in ques.7 above would you prepare or buy for consumption?

…………………………………………………………………………………

…………………………………………………………………………………

9. In preparing your meat product for consumption, which of the spices named in ques.2 above do you commonly use?

……………………………………………………………………………………………

……………………………………………………………………………………………

10. Which cooking methods do you employ in preparing the named product in ques. 9?

   a) Roasting b) Steaming c) Boiling d) Grilling

   e) Frying f) Broiling g) all

11. Why will you not use spices in a named cooking method (if you do)?

   Cooking method: …………………………………………………………………

   Reason……………………………………………………………………………

   …………………………………………………………………………………

12. Why do you use spices in your meat product preparations?

   a) Enhance flavor of food c) Make food more attractive

   b) To garnish the food d) Prevent spoilage of food

   c) To enhance keeping quality of food

   d) State others: …………………………………………………………

13. Do you encounter problem(s) in the use of spices?

a) Yes b) No

14. If yes, name the spice(s) in question

…………………………………………………………………………………………

…………………………………………………………………………………………

15. State the problem(s) you encounter

…………………………………………………………………………………………

…………………………………………………………………………………………

16. Do some spices need further preparations before use in meat preparations?

a) Yes b) No

17. If yes, which of them needs such preparations?

…………………………………………………………………………………………

…………………………………………………………………………………………

18. Give a brief description of how such preparation(s) are made

…………………………………………………………………………………………

…………………………………………………………………………………………

19. Does the preparation discourage you from the use of such spice(s)?

a) Yes b) No

20. If yes, what do you wish to be done to avoid such preparations?

…………………………………………………………………………………………

…………………………………………………………………………………………

21. In which form do you obtain your spices?

a) Whole form b) Ground form c) Paste d) Granular form

e) Powder

State others: ………………………………………………………………………

22. Where do you commonly obtain the spices you use in your meat preparations?

a) Home b) Farm c) Markets d) Special stores State others:

……………………………………………………………………………………

……………………………………………………………………………………

23. Which of the spices mentioned in ques.2 above are easy to obtain?

……………………………………………………………………………………

……………………………………………………………………………………

24. What is your average income (in Ghana Cedis) per month?

a) Below 100 b) 100- 500 c) 500 - 1000 d) above 1000

25. How much do you spend on spices (in Ghana Cedis) in a month?

a) 1-5 b) 5-10 c) 10-50 d) 50 above,

specify…………………………………………………..

26. What are your misgivings about the use of spices?

……………………………………………………………………………………

……………………………………………………………………………………

27. Do you think spices can cause any health problems?

a) Yes b) No

28 If yes, what type of health problems do you think can be encountered?

……………………………………………………………………………………

……………………………………………………………………………………

29. Which of the health problems have you encountered yourself?

……………………………………………………………………………………

……………………………………………………………………………………

APPENDIX 2b

QUESTIONNAIRES

Kwame Nkrumah University of Science and Technology
Department of Animal Science

<u>Meat Processor / Vendor's Questionnaire</u>

On

THE USE OF DIFFERENT SPICES IN KUMASI METROPOLIS *This questionnaire seeks to select responses from meat processors/meat product vendors on the use of spices in meat products. Kindly assist in providing accurate information on the questions raised by ticking the appropriate answers.*

SECTION A: PERSONAL DATA date ……………..………

1. Gender: (a) Male (b) Female
2. Age: (a) ≤25 b) 26-35 (c) 36-45 (d) 46-50 (e) above 50
3. Location …………………………………………………………………….
4. Tribe: ……………………………………………………………………………
5. Religion: (a) Christianity (b) Islam (c) Others
6. Marital Status: (a) Single (b) Married (c) Divorced (d) Widowed
7. Household Size: (a) less than 4 (b) 4-6 (c) above 6
8. Additional Occupation: (a) Civil Servant (b) Business / Trader
9. (d) others specify …………………………………………………………
10. Level of Education: (a) basic (b) Secondary (c) Tertiary (d) No formal **SECTION B**

1. Are you familiar with spices and their use?

   a) Yes b) No

2. If yes, which spices or herbs are you familiar with?
   a) Cinnamon i) Negro Pepper (*Hwentea*)

b) Chilies j) Mace

c) Coriander k) Cardamom

d) *Prekese* l) West African Black Pepper

e) Cloves m) Aniseed

f) Nutmeg n) Cumin

g) African Locust Bean (*Dawadawa*) o) Ginger

h) Grains Of Paradise (*Soro Wisa*) p) Calabash Nutmeg (*Awer amba*) q) State others:

…………………………………………………………………………………

…………………………………………………………………………………

3. Do you use spices in your meat preparations?

a) Yes b) No

4. If yes, which of the spices mentioned in ques.2 above do you commonly use?

…………………………………………………………………………………

…………………………………………………………………………………

Mention other not listed in ques.2 above

…………………………………………………………………………………

5. Which kinds of meat products do you use this/these spices?

b) Sausages b) Burgers c) Meat loaf d) Barbecue e) Khebab e) State others:

…………………………………………………………………………………

…………………………………………………………………………………

6. In which cooking methods do you employ the use of spices?

a) Roasting b) Steaming c) Boiling d) Grilling

e) Frying f) Broiling g) all

7. Why will you not use spices in a named cooking method (if you do)?

Cooking method:

Reason…………………………………………………………………………

…………………………………………………………………………………

8. Why do you use spices in your meat product preparations?

f) Enhance flavor of food c) Make food more attractive

g) Garnish the food d) Prevent spoilage of food

h) Enhance keeping quality of food

e) State others:

…………………………………………………………………………………

…………………………………………………………………………………

9. Do you encounter problem(s) in the use of spices?

a) Yes b) No

10. If yes, name the spice(s) in question

…………………………………………………………………………………

…………………………………………………………………………………

11. State the problem(s) you encounter

…………………………………………………………………………………

…………………………………………………………………………………

12. Do some spices need further preparations before use in meat preparations?

a) Yes b) No

13. If yes, which of them needs such preparations?

…………………………………………………………………………………

…………………………………………………………………………………

14. Give a brief description of how such preparation(s) are made

…………………………………………………………………………………

…………………………………………………………………………………

15. Does the preparation discourage you from the use of such spice(s)?

 a) Yes b) No

16. If yes, what do you wish to be done to avoid such preparations?

…………………………………………………………………………………

…………………………………………………………………………………

17. In which form do you obtain your spices?

 a) Whole form b) Ground form c) Paste d) Granular form f) Powder State others:

…………………………………………………………………………………

…………………………………………………………………………………

18. Where do you commonly obtain the spices you use in your meat preparations?

 a) Home b) Farm c) Markets d) Special stores b) State others:

…………………………………………………………………………………

…………………………………………………………………………………

19. Which of the spices mentioned in ques.2 above are easy to obtain?

…………………………………………………………………………………

…………………………………………………………………………………

20. What is the average production cost (in Ghana Cedis) per kilogram of your products?

 a) 1-10 b) 11 - 15 c) above 15

21. How much do you spend on spices per kilogram of product?

 a) 1-5 b) 6-10 c) 11-15 d) state any other………………

22. How much profit (in Ghana Cedis) do you make from the sale of a kilogram of the product?

a) 1-2 b) 2-3 c) 3-5 d) above 5

23. What are your misgivings about the use of spices?

…………………………………………………………………………………

…………………………………………………………………………………

24. Do you think spices can cause any health problems?

a) Yes b) No

25. If yes, what type of health problems do you think can be encountered?

…………………………………………………………………………………

26. Which of the health problems have you encountered yourself?

………………………………………………………………………………………

………………………………………………………………………………………

APPENDIX 2c

QUESTIONNAIRES

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF ANIMAL SCIENCE

SCORING SHEET FOR SENSORY EVALUATION OF FRANKFURTER SAUSAGES

USING A 9-POINTS HEDONIC SCALE

GENDER: ……….................................... **PANELIST NO**………………..

Instruction: Look, smell, chew and taste the given samples, then place an **x** mark on the point in the scale which best describes your feeling.

A. COLOUR/APPEARANCE

Score	Sample Code					
(**9**) Like Extremely						
(**8**) Like Very Much						
(**7**) Like Moderately						
(**6**) Like Slightly						
(**5**) Neither Like Nor Dislike						
(**4**) Dislike Slightly						

………………………………………………………………………………………

(**3**) Dislike Moderately						
(**2**) Dislike Very Much						
(**1**) Dislike Extremely						

B. AROMA

Score	**Sample Code**					
(**9**) Like Extremely						
(**8**) Like Very Much						
(**7**) Like Moderately						
(**6**) Like Slightly						
(**5**) Neither Like Nor Dislike						
(**4**) Dislike Slightly						
(**3**) Dislike Moderately						
(**2**) Dislike Very Much						
(**1**) Dislike Extremely						
State Specific Aroma Of Sample noticed						

Comment on specific ***spice aroma***……………………..…………………………………………

Comment if ***off -flavour***…………………………………………………………………………………

C. TASTE

Score	**Sample Code**					
(**9**) Like Extremely						
(**8**) Like Very Much						
(**7**) Like Moderately						
(**6**) Like Slightly						
(**5**) Neither Like Nor Dislike						
(**4**) Dislike Slightly						
(**3**) Dislike Moderately						

(**2**) Dislike Very Much						
(**1**) Dislike Extremely						

D. JUICINESS

Score	Sample Code					
(**9**) Extremely juicy						
(**8**) Very Much juicy						
(**7**) Moderately juicy						
(**6**) Slightly juicy						
(**5**) Neither juicy Nor dry						
(**4**) Slightly dry						
(**3**) Moderately dry						
(**2**) Very Much dry						
(**1**) Extremely dry						

E. TENDERNESS

Score	Sample Code					
(**9**) Extremely tender						
(**8**) Very Much tender						
(**7**) Moderately tender						
(**6**) Slightly tender						
(**5**) Neither tender Nor tough						
(**4**) Slightly tough						
(**3**) Moderately tough						
(**2**) Very Much tough						
(**1**) Extremely tough						

F. OVERALL ACCEPTABILITY

	Sample Code

Score						
(9) Extremely acceptable						
(8) Very Much acceptable						
(7) Moderately acceptable						
(6) Slightly acceptable						
(5) Neither accepted Nor unaccepted						
(4) Slightly unacceptable						
(3) Moderately unacceptable						
(2) Very Much unacceptable						
(1) Extremely unacceptable						

GENERAL COMMENT:

…………………………………………………………………………………………………

………………………………………………………………………………….........................