EVALUATION OF TETRAPLEURA TETRAPTERA (‘Preke’) EXTRACT ON THE SENSORY CHARACTERISTICS AND MICROBIAL QUALITY OF FRESH PORK SAUSAGE.

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MPHIL. ANIMAL SCIENCE DEGREE

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DECLARATION

I hereby declare that this thesis which is submitted to the Department of Animal Science, College of Basic and Applied Sciences, University of Ghana, for the award of Master of Philosophy in Animal Science degree is the result of my own investigation. This thesis has not been submitted or presented for another degree elsewhere, either in part or in whole, except for other people’s work which was duly cited and acknowledged.

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DEDICATION

This work is dedicated to my parents, Mr. J. T. Lartey and Mrs. Beatrice T. Lartey, my siblings and my daughters and sons in Christ.
ABSTRACT

The effect of varying concentrations of essential oil (EO) extracts from *T. tetraptera* pods on the sensory characteristics and microbial quality of fresh pork sausages were evaluated. Gas chromatography-mass spectrophotometry (GC-MS) analysis of the extract revealed thirty-two (32) chemical compounds which included organic acids, phenols and alcohols, including eugenol. Sausages were prepared from de-boned ham, divided into four batches and mixed with black pepper and garlic (0.5% each). Three batches were treated with three levels of *Tetrapleura tetraptera* extract (0.2%, 0.4% and 0.6%) and the fourth batch served as control (without *T. tetraptera*). Sausage samples were grilled for 35 minutes to an internal temperature of 71°C, cut into 2cm length and placed in air-tight disposable containers for evaluation. The test of organoleptic properties of cooked products showed that there were no significant differences (P˃0.05) between the *T. tetraptera* treated products and the control with respect to the juiciness, crumbliness and flavour. However, there was significant difference (P˂0.05) in palatability as the level of extract inclusion increased beyond 0.2%. There were no significant effects (P˃0.05) of the treatments on microbial counts in the products. Similarly, there was no significant effect (P˃0.05) of storage times (0, 3 and 6 days) at 4°C on microbial counts. *L. monocytogenes, S. enteric spp.* and *E. coli* were not susceptible to the *T. tetraplera at concentrations ≤1%*. 
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CHAPTER ONE

1.1 INTRODUCTION

Sausages are products in which comminuted meats are modified by various processing methods to alter organoleptic and storage properties. According to Marchello and Garden-Robinson (2004) sausages are one of the most famous meat products prepared from chopped or ground meat blended with spices or seasonings and stuffed into natural or artificial casings. Early on, Price and Schweigert (1971) had also reported on the distinctive flavour and aroma imparted by spices and other ingredients to sausages. Herbst (1995) also reported that spices are pungent or aromatic seasonings obtained from the bark, buds, fruits, roots, seeds or stems of various plants and trees. Spices may be added as whole seeds, coarsely ground, powdered, or in the form of oleoresin. Additionally, many spices and herbs are valued not only for their flavour and fragrance but also their antimicrobial activities and medicinal effects on some micro-organisms including food-borne pathogens (Shan et al., 2007). In most African countries, including Ghana, some indigenous plants and plant parts such as ‘Dawadawa’ (*Parkia biglobosa*), ‘Prekese’ (*Tetrapleura tetraptera*), ‘Akokobesa’ (Basil), ‘Nketenkete’ (*Desodium adscendens*) and ‘Hwentea’ (*Xylopia aethopica*) are used as whole or ground spices in the preparation of local foods.

In some parts of the world, production of sausages is complicated by religion, sentimental considerations, taboos and other customs. In some countries in South-East Asia, according to Savic (1985), cattle slaughter is not permissible while in other regions in Asia and some areas of Africa, pigs are not slaughtered. In some developing
countries, there are areas which frown on the consumption of certain parts of slaughtered animals.

According to reports by the Livestock Agripreneures (2014), pork has become quite popular with consumers in Ghana, particularly in the urban areas leading to a lot of farmers turning to pig production to meet the growing demand for meat and meat products.

Concerns about the safety of meat and meat products have instigated consumer advocacy towards a shift from the use of chemical/synthetic condiments to natural additives in processed meat.

In spite of the historical usage of indigenous plants and their parts in food preparation, little has been reported or documented on the effect of their incorporation in processed meat.

The purpose of this study, therefore, was to evaluate the effect of *T. tetraptera* (‘prekese’) as a spice on the sensory characteristics and microbial quality of fresh pork sausages.

### 1.2 AIMS OF PROJECT

The aim of this project is to:

i. Determine the effect of *T. tetraptera* pod extract on the sensory characteristics of fresh pork sausages.

ii. Evaluate the antimicrobial effects of *T. tetraptera* pod extract.
1.3 HYPOTHESIS

The use of *T. tetraptera* in fresh pork sausages formulation has no undesirable effect on the sensory characteristics and microbial count in products.
CHAPTER TWO

LITERATURE REVIEW

2.1 Types of Meat Products

Different types of meat products are manufactured to meet the taste of different people living at different locations. This ranges from finely chopped meat mixed with cereals, spices and other ingredients which is encased in the intestine of an animal or from a synthetic membrane (Pyke, 1981) to others such as marinated lean meat which are arranged around a skewer bar (khebab) and minced meat usually in disc-like shapes (hamburger) among others (Gunter and Hautzinger, 2007).

2.2 Sausages

Marchello and Garden-Robinson (2004) defined sausages as chopped or ground meat products that have been blended with spices or seasonings and stuffed into natural or artificial casings. The word sausage was derived from the Latin word *salsus* which means salted or preserved by salting, which dates back thousands of years ago (USDA/FSIS, 1999).

Cutting up scrap meats, salting and sealing it in casings made from the intestines and other organs of animals was one of the first discoveries of early pastoralist (Nordan, 2010). The art of sausage making has developed gradually from this simple process of salting and drying meat to the modern meat processing industry (Nordan, 2010). The procedure of stuffing meat into casings remains basically the same today, but sausage recipes have been greatly refined (Marchello and Garden-Robinson, 2004).
According to Marchello and Garden-Robinson (2004), sausage making evolved as an effort to economize and preserve meat that could not be consumed fresh at slaughter. Busboom and Field (2003) reported that some early sausage makers became so adept in spicing and processing sausages of distinctive types that the fame of their products spread throughout Europe. Sausages often bear the name of the city of origin: Milano, Romano, Genoa, Bologna and Salami from cities in Italy, Frankfurters from Frankfurt and Weinerwurst from Vienna, Austria (Busboom and Field, 2003).

2.3 Classification of Sausages

FAO (1985) categorized sausages into roughly two general groups, that is; the raw and the heat processed sausages. Raw sausages, which are not subjected to the process of smoking include air-dried sausages. Different types of sausages are created all over the world and each region has developed their own distinctive style of sausage influenced by the availability of local ingredient, casing and spices (Tronsky et al., 2004). Climate is a major factor for development of region specific fresh and dry sausages. In cold seasons raw sausages are able to keep under room temperature for a period of time without refrigeration. Dry sausages, which do not require any refrigeration, were created in warmer regions (Tronsky et al., 2004).

Sausages can be classified in a variety of ways depending on how they are processed:

Raw sausages are made from raw ground meats of any species of meat animals and poultry, seasoned and stuffed into casings or left in bulk form. Raw sausage is not cured or smoked; it must be fully cooked before eating. Examples are pork sausage; Italian; bulk pork sausage (Tronsky et al., 2004).
Other types of sausages are cooked and these are usually cured with salt and sugar and sometimes nitrite added, heated to an internal temperature of $65^\circ\text{C} - 70^\circ\text{C}$ during processing, and frequently smoked. They require refrigeration and can store for at least 2 weeks in unopened vacuum-sealed packages and for 1 week in non-vacuum packages. Examples include hotdogs and luncheon meats such as Bologna, Cottosalami, Polish sausage, Frankfurter and Braunschweiger (Busboom and Field, 2003). Additionally, uncooked, smoked sausages are made from meats which are ground, seasoned, stuffed into casings, smoked and must be fully cooked before eating. Examples are some kielbasas, mettwurst, teawurst and smoked country-style pork sausage (Tronsky et al., 2004). Furthermore, dry and semi-dry sausages are made from meat which are ground, seasoned, cured, stuffed into casings, fermented, often smoked and carefully air-dried but not cooked; stuffed into casings and allowed to ferment. Examples are pepperoni; German salami, Lebanon bologna, Genoa salami, thuringer and cervelat (Tronsky et al., 2004). Specialty sausages are a diverse category that may contain cured, uncured, smoked, and non-smoked meats that do not readily “fit” into the other categories; formed into loaves. Examples are olive loaf; head cheese; jellied corned beef; scrapple; souse (Tronsky et al., 2004).

2.4 Methods of Sausage Production

The grinding processes will vary according to the manufacturer and the nature of the product. Some sausage products use coarsely ground meat while others use more emulsified (USDA/FSIS, 1999). In general, it can be said that the finer the degree of grinding and chopping, the more complete the extraction of protein will be while the
spreading or slicing properties of the finished product will also be improved (FAO, 1985).

Sausage can be smoked and heated in order to pasteurize and prolong its shelf life, as well as to impart a smoky flavour and to improve its appearance (Marchello and Garden-Robinson, 2004). A few products, such as mettwurst, are smoked with a minimum of heating and are designed to be cooked before consumption. Others such as liver sausage are cooked but not smoked (Marchello and Garden-Robinson, 2004).

Apart from the organoleptic benefits, smoke constituents produce bacteriostatic and bactericidal actions during treatment such that at the end of the smoking process, the product’s microbial population is practically zero (Girard, 1992). Smoking can be done using liquid smoke, hardwood and sawdust. Cooking or scalding follows immediately after smoking. The temperature of water in cooking vats may be about 73-76°C. A final internal sausage temperature of 65°C is an optimum end-point temperature providing a sufficient shelf life of the product and desired organoleptic characteristics (FAO, 1985).

There are many methods of cooking sausages, according to FAO (1985).

1. Immersing in the cooking vat, hot showering that is conducted in a smokehouse equipped with shower nozzles or hot showering in separate hot water spray cabinets to which sausages are moved immediately after smoking.

2. Cooking by dry heat using the smokehouse temperature and giving only a final brief hot water shower or cooking in tight boxes into which hot steam is injected.
2.5 **Economic importance of Sausage production**

New product development is an essential ingredient in meat processing and ready meal market in a rapidly growing society. In most developed countries, there is an increasing demand for different tastes, forms of meat and meat products to accommodate the changing lifestyle of consumers (Harris, 2005). With sausage manufacturing techniques, animal by-products including trimmings of low economic value are re-valued through their use in sausage preparation, thus, contributing to economic and social development (FAO, 1985).

2.6 **Materials and composition of Sausages**

According to a report by Teye (2007), quality meat product can only be obtained from quality and hygienically produced raw materials. The FAO (1985) added that the selection of ingredients is basic for the production of sausages of uniform standard quality. Although beef, veal and pork are the main meat sausage materials, mutton, poultry and meat from other species of animals, together with edible by-products, are also of importance. Apart from meat, a number of non-meat ingredients, such as curing salts, sugar, spices and casings, are used in sausage production. Many countries have regulations which have an important bearing on the use of different ingredients in sausage formulations. However, in a number of developing countries, there are either no regulations or they are not enforced. In developed countries, there are governmental pressures to reduce the amounts of salt, nitrite, nitrate and other additives or ingredients in different kinds and types of sausages. Generally, sausages are made from red meat (beef, pork, lamb or veal), poultry or a combination of both (USDA, 2009).
The FAO (1985) had reported that meat used in raw sausages need careful trimming of fat and sinews, soft intramuscular fatty tissue in particular should be removed. Chilled meat is more suitable for both course and fine sausages as the meat batter can be more readily maintained at optimal temperatures (FAO, 1991). In processed meat product, fats are added to make the product softer and also to improve the flavour and taste (Gunter and Hautzinger, 2007).

2.6.1. Meat Ingredients

According to the FAO (1985), the principal meat ingredient used in the formulation of sausages is the skeletal muscle meats of slaughtered animals. The report continues to indicate that the different skeletal muscles vary not only in their fat, water and protein contents but also their water-binding and emulsifying properties, colour and the species of animal from which they originate among a few other characteristics.

2.6.2 Non-meat ingredients

Non-meat ingredients are used to impart flavour, inhibit bacterial growth and increase the yield of the sausage (Tronsky et al., 2004). According to USDA/FSIS (1999), there are many non-meat ingredients that are essential to sausage making process. These ingredients include water, spices, non-fatty milk, extenders and binders (Tronsky et al, 2004). Different spices and herbs have been added to various meat products, mainly to produce the characteristic flavour of the final product. Commonly used spices include ground pepper, paprika, garlic, mace and cardamom (Verluyten et al., 2004). Additives are usually substances which are not consumed as food by itself, but are added to develop certain technological and quality characteristics (Heinz and Hautzinger, 2007). They are included in sausages to improve upon the colour,
minimize rancidity or to inhibit microbial growth. Such additives include sodium nitrate, phosphate and sodium erythorbate (Tronsky et al, 2004).

2.6.2.1 Common salt (Sodium chloride)

It is a very common ingredient added to sausages. Apart from its role as a preservative, it is also important in imparting flavour and solubilizing protein (ILO, 1985). Salt serves three functions in the meat:

1. It lowers the amount of available water (which preserves or extends shelf-life),
2. It extracts the meat myofibrillar proteins needed to make the product bind and to emulsify fat, and
3. For flavour enhancement (National Livestock and Meat Board, 1991). The amount of salt used in sausage products varies depending on the geographical location and the desire of individual sausage processors. The salt content of most processed meat ranges from 2.5-5.0% of the final product (FAO, 1990).

Fermented sausages usually contain 3-5 percent salt, while fresh sausages contain 1.5-2.0 percent salt with the vast majority of cooked sausages containing 2-3% salt (Price and Schweigert, 1971). An acceptable level of salt in dry or semi-dry sausages is about 3%. However, higher and lower salt levels are often used (FAO, 1985). Matulis et al (1995) reported that the combination of 11.25% fat and 1.3% salt was the minimum level required for acceptable sensory attributes in frankfurters with a pH value of 6.0.

2.6.2.2 Sugar

It is used for flavour and to counter the harsh taste of salt. It is also added as a medium for the microbial fermentation process used to reduce the pH of dry and semi-dry
sausages (e.g. pepperoni). The lactic acid produced by fermentation of the sugar reduces the meat pH and gives these sausages their characteristic tangy flavour (National Livestock and Meat Board, 1991).

Substantial amounts of sugars are particularly common in Asian style traditional products (up to 8%), where they are instrumental in lowering water activity $a_w$ and extending the shelf-life (Gunter and Hautzinger, 2007).

### 2.6.2.3 Water

According to Tronsky *et al.* (2004), water and ice are added to provide moisture and keep the sausage cold. Cold temperature delays microbial growth and also ensures a better final product texture. Ice and water can also be added to increase the yield of sausage, but there are upper limits for wholesale or retail marketing. Water also aids in dissolving salt to facilitate its distribution within the meat. Texture and tenderness of the finished sausages are markedly affected by added water content (Pearson and Gillet, 1999).

### 2.6.2.4 Other essential items - Casing

The term casing refers to the envelope enclosing an animal product, mainly sausages (Bradley, 2002). Casings may be natural (derived from intestines and sometimes stomach of animals) or artificial (collagen manufactured from animal by-products, mainly cattle by an industrial process).

#### 2.6.2.4.1 Natural casings (Edible Collagen Casing)

Natural casings are almost exclusively prepared from different parts of the alimentary canal of pigs and ruminants. Pig casings are derived from the stomachs, small
intestines (pig casings, smalls or rounds), large intestines (caps and middles) and terminal straight end of the large intestines (bungs) (FAO, 1985). Cattle casings are obtained from the oesophagus (weasands), small intestines (rounds or runners), caecum (bungs), large intestines (middles) and urinary bladders. Only the small intestines of sheep are used for sausage casings (FAO, 1985). According to Busboom and Field (2003), natural casings are not as uniform or as easy to use, so commercially they are used primarily for gourmet sausages.

2.6.2.4.2 Artificial casing

Heinz and Hautzinger (2007) reported that artificial casings were developed at the beginning of the 20th century when, in some countries, the supply of natural casings could no longer cope with the demand for such natural casings from the growing meat industries. Following the development of highly automated sausage filling equipment, artificial casings proved to be better suited to those systems, mainly due to their uniformity.

Artificial casings offer a uniform cylindrical shape and the choice of any specific diameter and suitable tensile strength as well as resistance to damage. The artificial casings are made from cellulose, collagen, plastic and other materials (FAO, 1985), and can be stored for longer periods of time and require less preparation prior to use (Tronsky et al., 2004).

2.7 Meat Spoilage and Related Micro-organisms

Muscles of healthy animals are presumed sterile until slaughtering and butchering processes provides bacteria with an opportunity to colonize meat surfaces.
Contamination of meat is a continuing possibility from the moment of bleeding until consumption (Olaoye, 2011). In the abattoir, potential sources of microbial contamination range from hide and hair to soil adhering thereto, gastro-intestinal tract content (released during dressing), air and water (used for washing the carcass and for cleaning floors and equipments), among others (Holzapfel, 1998; Stanbridge and Davis, 1998).

Table 1: Some typical microbial counts from sources of microbial contamination in an abattoir, at incubation temperature of 20ºC (Lawrie and Ledward, 2006).

<table>
<thead>
<tr>
<th>Source</th>
<th>Bacteria</th>
<th>Yeast</th>
<th>Moulds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hides (cfu/g)</td>
<td>$3.3 \times 10^6$</td>
<td>580</td>
<td>850</td>
</tr>
<tr>
<td>Surface soils (cfu/g)</td>
<td>$1.1 \times 10^5$</td>
<td>$5 \times 10^4$</td>
<td>$1.2 \times 10^5$</td>
</tr>
<tr>
<td>Gastrointestinal contents: Faeces (cfu/g)</td>
<td>$9.0 \times 10^7$</td>
<td>$2.0 \times 10^5$</td>
<td>$6.0 \times 10^4$</td>
</tr>
<tr>
<td>Gastrointestinal contents: Rumen (cfu/g)</td>
<td>$5.3 \times 10^7$</td>
<td>$1.8 \times 10^5$</td>
<td>1600</td>
</tr>
<tr>
<td>Airborne contamination (no. deposited from air/cm²/hr) (cfu)</td>
<td>140</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Water used on slaughter floors (cfu/ml)</td>
<td>$1.6 \times 10^5$</td>
<td>30</td>
<td>480</td>
</tr>
<tr>
<td>Water present in receptacles from immersion cloths (cfu/ml)</td>
<td>-</td>
<td>$1.4 \times 10^5$</td>
<td>-</td>
</tr>
</tbody>
</table>

Meat spoilage usually results from the metabolic activity of a variety of microorganisms. The microbial flora involved in the decomposition of specific muscle tissues depends on the characteristics of the meat (residual glucose, pH), the
environment in which meat is stored (temperature, atmosphere), as well as the O₂ permeability of packaging material used, the number of bacteria initially present, and the ability of bacteria present to grow (Garcia-Lopez et al., 1998).

Apart from hygiene and storage temperature, the acidity of the meat and the structure of the muscular tissue also affect the rate of meat spoilage. For example, liver will decompose faster than the firm muscular tissue of beef (Berkel et al., 2004). The glycogen content of animal muscles is reduced when the animal is exposed to pre-slaughter stress. This changes the pH of the meat, either to higher or lower levels, depending on the production level of lactic acid (Miller, 2002; Chambers and Grandin, 2001; Rahman, 1999a). High pH (6.4-6.8) caused by long term stress results in Dark, Firm and Dry (DFD) meat, consequently, rendering its shelf life shorter (Miller, 2002; Chambers and Grandin, 2001). Severe short term stress results in a pale, soft and exudative (PSE) meat. PSE meat has a pH lower than normal ultimate value of 6.2 which is responsible for the breakdown of proteins, providing a favorable medium for the growth of bacteria (Miller, 2002; Chambers and Grandin, 2001; Rahman, 1999a).

2.8 Meat Preservation

The wide range of nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens. It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality (Aymerich et al., 2008). All methods used in meat preservation are principally concerned with inhibiting microbial spoilage, although
other methods of preservation are sought to minimize other deteriorative changes such as colour and oxidative changes.

However, a number of interrelated factors influence the shelf life and keeping quality of meat, specifically holding temperature, atmospheric oxygen ($O_2$), endogenous enzymes, moisture (dehydration), light and most importantly, micro-organisms. All of these factors, either alone or combined, can result in detrimental changes in the colour (Faustmann and Cassens, 1990), odour, texture and flavour of meat. Although meat spoilage can occur in the absence of micro-organisms (e.g., proteolysis, lipolysis and oxidation), microbial contamination is by far the most crucial factor in keeping quality of fresh meat (Lambert, et al., 1991).

As a result of the rise in interest in bio-preservation of food systems, new natural antimicrobial compounds from different origins are being developed. A variety of treatments in the prevention of food spoilage have been investigated, including animal-derived systems (lysozyme, lactoferrin, and magainins), plant-derived products (phytoalexins, herbs, and spices), and microbial metabolites (bacteriocins, hydrogen peroxide, and organic acids) (Lavermicocea et al., 2003). The use of antibiotics in food preservation, on the other hand, is increasingly being discouraged because of concerns relating to the emergence of drug-resistant strains and chronic toxicity that have arisen as a result of their abuse. In addition, illnesses such as diarrhoea caused by loss of normal intestinal micro biota, resulting from antibiotic misuse are escalating (Park et al., 2005).

While a particular method of preservation may involve several antimicrobial principles, the famous traditional meat preservation methods such as drying, smoking,
brining, fermentation, refrigeration and canning which use the principle of temperature and moisture control, are being replaced by more directly inhibitory methods (bactericidal and bacteriostatic, such as ionizing radiation), non-thermal inactivation technologies such as high hydrostatic pressure (HHP), new packaging systems such as modified atmosphere packaging (MAP) and active packaging (AP), natural antimicrobial compounds and bio-preservation methods which attempt to be mild, energy saving, environmentally friendly and guarantee natural appearance while eliminating pathogens and spoilage micro-organisms (Zhou et al, 2010).

2.9 Spices and their uses

Price and Schweigert (1971) stated that spices impart distinctive flavour and aroma to meat products and also possess anti-oxidant properties. They may be added as whole seeds, coarsely ground, powdered, or in the form of oleoresin. Oleoresins are derived by solvent extraction of spices (Kramilch et.al, 1973). Though they have always been used to flavour food and drinks throughout the world, spices have also been used for crowning emperors, manufacture of medicines and perfumes, religious ceremonies and as burial accoutrements for the wealthy. Examples of spices include garlic, cinnamon, pepper, etc. (Herbst, 1995). Appendix 5 shows a table of common seasonings used in processed meat (Heinz and Hautzinger, 2007).
Table 2: Common seasonings used in processed meat (Source: Heinz and Hautzinger (2007))

<table>
<thead>
<tr>
<th>A. SPICES</th>
<th>Black/white pepper</th>
<th>Fruits seed Used in a variety (almost all) meat products (1–2.5g/kg).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paprika (Fruit seed)</td>
<td>Used in frankfurters, minced specialties and other products. Sometimes used as a colouring agent (1-5g/kg).</td>
<td></td>
</tr>
<tr>
<td>Chilli (Fruit seed)</td>
<td>For spicy products.</td>
<td></td>
</tr>
<tr>
<td>Pimento (Fruit seed)</td>
<td>It has an aroma similar to a mixture of nutmeg, cinnamon and cloves. Used in a variety of sausage products. Sometimes used as a partial replacement for black pepper in frankfurters and some smoked products (0.3-3.0g/kg).</td>
<td></td>
</tr>
<tr>
<td>Mace (Flower)</td>
<td>Used in liver sausages, frankfurters and bologna and similar (0.4-1.0g/kg)</td>
<td></td>
</tr>
<tr>
<td>Ginger (Rhizome)</td>
<td>Used in frankfurters and similar products (0.3-0.5g/kg)</td>
<td></td>
</tr>
<tr>
<td>Nutmeg (Fruit seed)</td>
<td>Used in bologna and minced ham sausages, frankfurters, liver sausage and gelatinous meat mixes (0.3-1.0g/kg)</td>
<td></td>
</tr>
<tr>
<td>Clove (Flower)</td>
<td>Used in bologna, gelatinous meat mixes and in blood and liver sausage (0.3-0.5g/kg)</td>
<td></td>
</tr>
<tr>
<td>Cinnamon (Bark)</td>
<td>Astringent and sweet, used in some countries in mortadella and bologna sausage (0.1-0.2g/kg)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. AROMATIC SEEDS</th>
<th>Cardamom</th>
<th>Rapid loss of aromatic constituents during storage. Used in liver sausage and gelatinous meat mixes (0.3-5.0g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celery seed</td>
<td>Used in fresh pork sausages (0.3-2.0g/kg)</td>
<td></td>
</tr>
<tr>
<td>Coriander seed</td>
<td>Contains about 13% of fatty matter and a trace of tannin. It is used in frankfurters, minced ham, luncheon meat (0.3-1.0g/kg)</td>
<td></td>
</tr>
<tr>
<td>Cumin</td>
<td>Used for meat specialties with distinct flavour (0.2-0.3g/kg)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. CONDIMENTAL HERBS</th>
<th>Marjoram</th>
<th>Used in liver and white raw-cooked sausages and gelatinous meat mixes. (0.5-2.0g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyme</td>
<td>For meat specialties with distinct flavour (0.2-0.3g/kg)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. CONDIMENTAL VEGETABLES</th>
<th>Onion (Bulb)</th>
<th>Used in liver sausage, gelatinous meat mixes meat loaves. Sometimes replace garlic (2.0-10.0g/kg).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic (Bulb)</td>
<td>Used in many types of raw-cooked sausages (0.1-0.2g/kg)</td>
<td></td>
</tr>
</tbody>
</table>
2.9.1 Black Pepper

According to a report by Anandarag (2015), black pepper (*Piper nigrum* L.) from the family Piperaceae, is a perennial vine grown for its berries extensively used as a spice and a medicine. It grows successfully between 20° North and South latitude, and from sea level up to 1500 m above sea level. It tolerates temperatures between 10°C and 40°C. The ideal temperature is 23°C-32°C with an average of 28°C. Optimum soil temperature for root growth is 26°C-28°C and an annual rainfall of 125-200 cm. It can be grown in a wide range of soils with a pH of 5.5 to 6.5, though in its natural habitat it thrives well in red laterite soils. It has been found to contain about 250 volatile oils (BACIS, 1999; Sumathykutty *et al.*, 1999; Menon *et al.*, 2000; Tewtrakul *et al.*, 2000) including monoterpenes, minor aldehydes and pyrazines. However, a report by Shahin *et al.* (2012) revealed 18 compounds from Bangledeshi Black pepper which included α-Pinene (16.685%), Caryophyllene (18.393%) and D-Limonene (16.168%) and 14 similar compounds from Indian Black Pepper.

2.9.2 Garlic

*Allium sativum* is a vegetable species that can be classified as either a food or a medicinal herb. It is a widely used plant product that is cultivated all over the world. Garlic belongs to the family *Amaryllidaceae* and the genus *Allium*. Its closest relatives in the onion genus include the onion, shallot, leek, rakkyo and chive (Block, 2010). The bulbs are mainly composed of water (approximately 84.09%), organic matter (13.38%) and inorganic matter (1.53%). The leaves consist of more or less the same components with slightly different ratios (water 87.14%, organic matter 11.27% and inorganic matter 1.59%) (Bilyk and Sapers, 1985; Abdel-Fattah and Edrees, 1972).
Allicin has been found to be the compound most responsible for the "hot" sensation of raw garlic (RG) (Macpherson et al., 2005). Allicin, along with its decomposition products diallyl disulphide and diallyl trisulphide, are major contributors to the characteristic odour of garlic, while other allicin-derived compounds, such as vinyldithiins and ajoene show beneficial in vitro biological activity (Block E., 2010), hence, its use as a spice in food. In addition, newer research has characterized some polar compounds of phenolic and steroidal origin, which proffer various pharmacological properties. These latter compounds, in contrast to the thiosulfinates, are without odor, and are also heat stable (Lanzotti, 2006). Furthermore, some of the scavenger properties of garlic are not affected by heating or cutting (Pedraza-Chaverri et al., 2006).

2.9.3  *T. tetraperta*

The ‘Prekese’ plant, scientifically called *Tetrapleura tetraptera* is from the family of *Mimosaceae* and commonly known as Aridan (fruit) in Nigeria. It is a single-stemmed, robust, perennial tree of about 30m. It has a grey/brown, smooth/rough bark with branches. The flower is yellow/pink and racemes white. The fruit has dark brown, four winged pods (12–25cm x 3.5–6.5cm). It is generally found in the lowland forest of tropical Africa. The fruit consists of a fleshy pulp with small, brownish-black seeds. The fruit possesses a fragrant, characteristically pungent aromatic odour, which acts as an insect repellent and a spice in foods (Aladesanmi, 2007). The dry fruit has a characteristic aroma which makes it a popular seasoning spice in Southern and Eastern Nigeria (Essien et al., 1994; Adesina, 1982; Okwu, 2001).
The fruit shell, fruit pulp and seed contain varying amounts of nutrients such as protein, lipids and minerals which are comparable to and some are even higher than popular spices and ginger (Essien et al., 1994). It is also a source of minerals e.g. calcium, phosphorus, potassium, zinc and iron. The plant has many traditional uses, mainly in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma and hypertension (Ojewole and Adesina, 1982). The ethanol extract and saponins from the bark of the stem exerts an inhibitory effect on luteinizing hormone released by pituitary cells, suggesting its use as contraceptive agent (El-Izzi et al., 1990). It is used extensively in soups for nursing mothers to prevent post-partum contractions (Nwawu and Akali, 1986) and gastro-intestinal disorders especially stomach ulceration (Noamesi et al., 1992)

2.9.3.1 Nutritional importance of *T. tetraperta*

The fruit shell, fruit pulp and seed contained varying amounts of nutrients such as protein, lipids and minerals which are comparable and some are even higher than popular spices and ginger (Essien et al., 1994). Okwu (2003) reported that the proximate composition of *T. tetraperta* as follows: crude protein (7.44% - 17.50%), crude lipid (4.98% - 20.36%), crude fibre (17% - 20.24%), carbohydrate (43.18% - 49.06%) and food energy (234.18% - 42,379.48 gl Cal). The species is also a source of minerals e.g. calcium, phosphorus, potassium, zinc and iron. In Ghana, it is used as a source of vitamins in diets (Okwu, 2003).

According to Abii and Amarachi (2007), it was observed that the dry fruit of *tetrapleura tetraperta* contains 9% ash, 4% oil and 3% moisture.
2.9.3.2 Medicinal uses of *Tetrapleura tetraptera*

The plant has many traditional uses, mainly in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma and hypertension (Ojewole and Adesina, 1983). The ethanol extract and saponins from the bark of the stem exerts an inhibitory effect on luteinizing hormone released by pituitary cells, suggesting its use as contraceptive agent (El-Izzi *et al*., 1990). It is used extensively in soups for nursing mothers to prevent post-partum contractions (Nwawu and Akali, 1986) and gastro-intestinal disorders especially stomach ulceration (Noamesi *et al*., 1992). Phytochemical screening revealed the presence of tannins phenolic compounds, saponins, alkaloids, steroids and flavoniods which are assumed to be responsible for its varied biological and pharmacological properties (Okwu, 2003).

2.9.3.3 *T. tetraptera* Availability

*T. tetraptera* is common on the fringe of the West African rainforest belt. Trees are widespread in the forests of tropical Africa, especially secondary forest. The species is found throughout the high forest zone, in the southern savannah-woodland and in the forest outliers of the African plains (Orwa *et al*., 2009). The tree is deciduous, losing its leaves in December. Flowering begins towards the end of February and ends in early April. The indehiscent pods mature and ripen from September to December. When the pods fall, their scent attracts rodents, which probably disperse the seeds (Orwa *et al*., 2009).
2.10 Essential Oils

Essential oils are volatile, natural, complex compounds composed mainly of terpenes in addition to some other non-terpene components, characterized by a strong odour that are formed by aromatic plants as secondary metabolites (Bakkali et al., 2008; Edris, 2007). Essential oils are composed of lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300, which can be physically separated from other plant components or membranous tissue (Protzen, 1993; Grassmann and Elstner, 2003; Schmidt, 2010; Sell, 2010).

The pharmaceutical properties of aromatic plants are partially attributed to essential oils (Edris, 2007). They represent a “green” alternative in the nutritional, pharmaceutical, and agricultural fields due to reported antimicrobial, antiviral, nematicidal, antifungal, insecticidal, and antioxidant properties (Akhtar and Mahmood, 1994; Dorman and Deans, 2000; Zygadlo and Juliani, 2000; Papachristos et al., 2004; Cavanagh, 2007; Podsdek et al., 2009; Adorjan and Buchbauer, 2010; Dandlen et al., 2010; Ntalli et al., 2010; Lang and Buchbauer, 2012), or even activities stimulating the nervous system (Buchbauer and Jirovetz, 1994; Edris, 2007; Heuberger 2010). Essential oils have been suggested as antioxidants and preservatives in food (Burt, 2004; Lanciotti et al., 2004; Fisher and Phillips, 2008; Tiwari et al., 2009) and incorporated into foodstuff packaging materials (Kuorwel et al., 2011). Therefore, in recent times, essential oils have gained great popularity as consumers have developed a particular ever-growing awareness toward the use of natural ingredients, especially in food, household and cosmetic products (Yamamoto, 2008; Jiang et al., 2011). As a matter of fact, trade quantities of a couple of popular oils such as eucalyptus or lemon
by far exceed 1000 metric tons per annum with an estimated value of several hundred million euros in 2007 (Brud, 2010; Franz and Novak, 2010).

Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopoeia (Bakkali et al., 2008). Several techniques can be used to extract essential oils from different parts of the aromatic plant, these include the use of liquid carbon dioxide or microwaves, water or steam distillation, solvent extraction, expression under pressure, supercritical fluid and subcritical water extractions (Bakkali et al., 2008; Edris, 2007).

2.1.1 Composition of Essential Oils

Essential oils are very complex natural mixtures which can contain about 20-60 components at quite different concentrations (Bakkali et al., 2008). Blitzke (2009) reported that a multitude of different, but often structurally closely related, components have been identified in essential oils and each oil in turn can be composed of only a few, up to a complex mixture of far more than 100 single substances, respectively. Flavour contribution of single compounds though does not strictly depend on their respective concentration but relies on the specific odour threshold that is determined by structure and volatility. Consequently, even minor components deriving from oxidation or degradation reactions may have a strong impact on the flavor if their aroma value is high enough (Grosch, 2007). For the most part, essential oil components can be assigned as lipophilic terpenoids, phenylpropanoids, or short-
chain valiphatic hydrocarbon derivatives (Kubeczka, 1979) of low molecular weight, with the first being the most frequent and characteristic constituents (Treibs and Merkel, 1960). Among these, allylic, mono-, bi-, or tricyclic mono- and sesquiterpenoids of different chemical classes make up the major part in essential oils, such as hydrocarbons, ketones, alcohols, oxides, aldehydes, phenols, or esters (Turek and Stintzing, 2013).

2.10.2 Essential Oil Composition of *T. tetraptera*

Udourioh and Etokudoh (2014) reported forty-four compounds representing 98.5% of the essential oil from the dry pods, using gas chromatography mass spectrophotometry (GC-MS). The predominant chemical constituents of the oil were acetic acid (34.59%), 2-hydroxy-3-butanone (18.25%), butanoic acid (8.35%), 2-methyl butanoic acid (7.58%), 2-methyl butanol (7.45%), butanol (4.30%), 2-methyl butanoic acid (3.65%), nerol (3.25%), 2-methyl butenoic acid ethyl ester (2.70%), 2-methyl butanoic acid ethyl ester (2.09%) and linalool (1.84%). The oil is dominated by carboxylic acid which gives an entirely different report from that of essential oil results obtained from other spices by Onyenekwe et al. (1997), Karioti et al. (2004), Ekwenye and Okorie (2010) and Abugri and Pritchett (2013). Terpene constituents which often dominate most essential oils as observed in other spices were detected as minor or trace constituents in *T. tetraptera* (Udourioh and Etokudoh, 2014). For example, β-caryophellene was 0.1%, α-pinene (0.1%), β-pinene (0.2 %), myrcene (0.09 %), γ-terpinene (0.2 %), whereas reports on essential oils of *Piper guineense* showed β-caryophellene (20.8 %), β-pinene (12.15%), α-pinene (10.6%), myrcene (1.8%) and γ-terpinene (4.9%) (Karioti et al., 2004; Ekwenye and Okorie, 2010; Abugri and
Pritchett, 2013). Linalool constitutes 1.8% of the essential oils and this account for the pepperish nature of the plant (Udourioh and Etokudoh, 2014).
CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Locations

*T. tetraptera* pods were obtained from the Madina Market, Accra. Extraction of essential oils from *T. Tetraptera* was carried out at the Chemistry Laboratory, University of Ghana. Thin layer chromatography (TLC) and gas chromatography-mass spectrophotometry (GC-MS) analysis of the extract was carried out at the University of Ghana and the Ghana Standards Authority, Accra, respectively. Pork was obtained from the Livestock and Poultry Research Centre and processed into sausages at the Meat Laboratory of the Animal Science Department, University of Ghana. Microbial isolates used for susceptibility test were obtained from the Microbiology Department, Animal Research Institute. Isolation and enumeration of target bacteria were done at the University of Ghana Microbiology Laboratory.

3.2 Thin Layer Chromatography (TLC)

Using TLC pipettes, spots of the extract were applied to TLC plates (10cm x 4.5cm), 2cm from the bottom. The plates were placed into a chamber with ethanol (96%), leaning them against the side of the chamber. A capillary action was observed until the solvent rose to a level, 0.5cm from the top. The plates were removed, air dried and observed under UV light. Components of the extract were delineated with circles using a pencil.
3.3 Gas chromatography- mass spectrophotometry (GC-MS) analysis

The extract was analyzed for essential oil components using a GC/MS Saturn 2200 and CP-3800 gas chromatograph with a Db-5 fused silica column (30m x 0.25μm x 0.25nm) and an MS mass range of 30°C-450°C EI. The injector temperature was 220°C with a splitless injection of 2μL and the carrier gas, helium, had a flow rate of 1ml/min. The total run time for the set-up was 25min.

3.4 Experimental Design

A 4x3 factorial design with 4 levels of inclusion of *T. tetraptera* extract (0, 0.2%, 0.4% and 0.6%) and storage time (0, 3 and 6 days) in 3 replicates under refrigeration condition was used in this study.

3.5 Essential oils Extraction

Seeds were removed from the *T. tetraptera* pods (500g) after crushing them. The seed-free crushed pods were sun-dried, ground and soaked in 1 litre of ethanol (96%) in a 2000ml conical flask. The ethanol-extract mixture was filtered off and the residue re-soaked. This protocol was repeated every four days for 3 weeks. The ethanol was distilled off the mixture using a Rotary Evaporator (Model R210, Switzerland) operated under 60°C temperature for 25 minutes to obtain a concentrated extract of *T. tetraptera*. The ethanol-soaked weight and volume of the resulting extract were determined and stored in a refrigerator until use.
3.6 Sausage Preparation

De-boned ham (2.3kg) was thawed overnight at a temperature of 1°C, cut into smaller sizes and minced using a 5mm grinding plate fixed to a table top mincer (Talleres Rommon, Spain). The minced meat was mixed with black pepper and garlic powder (0.5% inclusion for each) and divided into four batches. Three batches were treated with \textit{T. tetraptera} extract in varying levels (0.2%, 0.4% and 0.6%) respectively with the forth batch serving as the control with no \textit{T. tetraptera} extract (0%). Mixing was done manually by hand and done thoroughly to ensure homogeneity. The mixtures were extruded into natural casing using a hydraulic stuffer (Talleres Rommon, Spain). The sausage was linked into 3-inch lengths and stored under refrigeration (4°C) for sensory evaluation.

3.7 Measurements

3.7.1 Microbial Analysis

3.7.1.1 Aerobic plate count

Aerobic plate count was carried out using total plate count agar. The medium was autoclaved and maintained at 46°C. One gram of each sample was blended with 450µl of saline solution (0.85%) and 1:10 serial dilutions were made ($10^{-1}$-$10^{-5}$). 100µl of each dilution factor was transferred onto petri dishes (4 inch diameter) and molten agar (15-20 ml) was poured on them. Plates were gently swirled to uniformly mix the sample and incubated at 37°C for 24 hours. Colonies were counted from the plates and recorded.
3.7.1.2 Yeast and mould

Yeast and mould were isolated and enumerated using potato dextrose agar reagent, following the same protocol as previously indicated for aerobic plate count.

3.7.2 Anti-microbial susceptibility test:

Muellar-Hinton agar was reconstituted and 20ml aliquot into universal bottles and sterilized by autoclaving. Three bottles (corresponding to the number of test organisms) were poured into 9cm petri dishes and allowed to cool and set.

The selected test organisms were purified by sub-culturing unto nutrient agar, plated out to obtain discrete colonies and incubated at 37 ºC overnight.

The inoculums were prepared by selecting and adding 3-5 colonies of the test organisms to 5ml of 0.1% sterile peptone water and emulsified. The inoculum was compared with 1McFarland standard solution and incubated at 37ºC for about 10-15mins in an incubator.

From the extract (T. tetraptera) stock, various dilutions were prepared (0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1%).

The Muellar-Hinton sensitivity medium plates were dried for 10-15mins in an incubator and an aliquot of 1ml of each inoculum was spread on their corresponding plates. The set-ups were allowed to dry for 2-3mins for the organisms to seed onto the medium. Using a sterile perforator (13mm diameter), seven wells were made on the plates. 250µl of the extract prepared at different concentrations was dispensed into each of the six wells. A control was added using distilled water. The plates were incubated overnight at 37ºC.
Using the agar diffusion method, zone of inhibition of the various concentrations of the extract were measured at three different angles to the nearest millimeter using a ruler and the mean computed.

Mean zone of inhibition <2mm was interpreted as resistance of the test organism to the extract, zone of inhibition from 2mm to 2.9mm was interpreted as intermediate resistance and ≥3mm would indicate that the organism was susceptible to the extract.

3.7.3 Sensory Analysis

A total of six (6) panelists, comprising staff and students of the University of Ghana - Legon, were selected and trained to form a taste panel to determine the organoleptic attributes (crumbliness, juiciness, palatability and off-odours) associated with the respective treatments. The panelists were selected based on their familiarity with meat and the meat products intended to be evaluated, willingness to participate in the evaluation and willingness to be screened based on their ability to perceive the above stated sensory related attributes of meat products. Panelists were trained and products such as avocado, apple and chewing sponge were used as anchors for tenderness. Similarly, coconut pulp, copra and milk powder were used as anchors for juiciness and cookies, copra and bread as anchors for crumbliness. Lastly, dry smoked fish blended in water was used for rancid flavours and fresh fish exudates for fishy flavours. The sausage samples were grilled for 35 minutes to an internal temperature of 71°C, cut into lengths of approximately 2cm and put in air-tight disposable containers for tasting. Measurement of organoleptic properties was done over a ten-point hedonic scale.
3.8 Data Analysis

The data was compiled using Microsoft Excel and analysed using the proc mixed tool of SAS, Version 9.1 (SAS Institute, Cary, NC, USA). Means were considered significantly different at P≤0.05.
CHAPTER FOUR

RESULTS

4.1 Gas chromatography-mass spectrophotometry

GC-MS analysis outlined thirty two (32) compounds from the extract according to their retention time. Among these were organic acids, phenols and alcohols, including eugenol.

4.2 Sensory Analysis

Table 3 shows the means for the panelists’ response to the sensory characteristics of the products on a hedonic scale.

Table 3: Means for panelists’ responses based on the sensory parameters (Mean ± standard error).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crumbliness</td>
<td>8.49±0.64</td>
<td>8.25±0.64</td>
<td>8.27±0.67</td>
<td>8.96±0.65</td>
<td>ns</td>
</tr>
<tr>
<td>Juiciness</td>
<td>7.38±0.61</td>
<td>7.57±0.61</td>
<td>6.81±0.63</td>
<td>6.62±0.61</td>
<td>ns</td>
</tr>
<tr>
<td>Palatability</td>
<td>11.80±1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.12±1.35&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.78±1.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.10±1.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>*</td>
</tr>
<tr>
<td>Off-odour</td>
<td>3.60±1.37</td>
<td>3.71±1.37</td>
<td>3.60±1.37</td>
<td>2.76±1.37</td>
<td>ns</td>
</tr>
</tbody>
</table>

Means on the same row with different superscripts are significantly different. T<sub>1</sub> = Treatment 1 (with no extract), T<sub>2</sub>=Treatment 2 (0.2% extract), T<sub>3</sub>=Treatment 3 (0.4 % extract), T<sub>4</sub>=Treatment 4 (0.6% extract); ns = not significant; * = significant at P≤0.05; Crumbliness = How easily product disintegrates; Juiciness = How much moisture is elicited from product; Palatability = Overall acceptability of the product; Off-odour = any other odour apart from that of fresh meat.

The treated products were not significantly different (P>0.05) from the control with respect to crumbliness, juiciness and off-odour. However, there was a significant difference (P≤0.05) between the palatability of the treated products and the control.
4.3 Microbial Quality Analysis

Results for the microbial analysis (Table 4) of the products indicated that means for counts of the organisms (E. coli, aerobic plate count and yeast and mould) were not significantly different (P>0.05).

**Table 4:** Log CFU/g of aerobic, yeast and mould and E. coli counts.

<table>
<thead>
<tr>
<th>Microbe</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>4.83±0.33</td>
<td>4.68±0.33</td>
<td>5.17±0.33</td>
<td>4.47±0.33</td>
<td>ns</td>
</tr>
<tr>
<td>Y/M</td>
<td>5.21±0.33</td>
<td>5.23±0.33</td>
<td>5.18±0.33</td>
<td>4.94±0.33</td>
<td>ns</td>
</tr>
<tr>
<td>E. coli</td>
<td>3.93±0.33</td>
<td>3.96±0.33</td>
<td>3.95±0.33</td>
<td>3.91±0.33</td>
<td>ns</td>
</tr>
</tbody>
</table>

Means on the same row with different superscripts are significantly different (P≤0.05); T1 = Treatment 1 (with no extract), T2=Treatment 2 (0.2% extract), T3=Treatment 3 (0.4 % extract), T4=Treatment 4 (0.6% extract); ns = not significant; APC = Aerobic plate count; Y/M=Yeasts and Moulds; E. coli = Escherichia coli.

Table 5 shows means for counts over the three storage days (0, 3 and 6). The results revealed that storage time (up to 6 days) did not affect microbial count significantly (P>0.05).

**Table 5:** Log CFU/g of aerobic, yeast and mould and E. coli counts (Comparison at storage times, 0, 3 and 6 days at 4 ºC)

<table>
<thead>
<tr>
<th>DAYS</th>
<th>MICROBE</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APC</td>
<td>4.83±0.17</td>
<td>4.94±0.15</td>
<td>4.68±0.15</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>3.81±0.15</td>
<td>4.18±0.15</td>
<td>3.83±0.15</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Y/M</td>
<td>5.13±0.31</td>
<td>5.23±0.31</td>
<td>5.06±0.31</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns = not significant; APC = Aerobic plate count; Y/M=Yeasts and Moulds; E. Coli = Escherichia Coli.
4.4 Anti-microbial Susceptibility

Serial dilutions of *T. tetraperta* extract (0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1%) were tested against three food-borne pathogens (*Listeria monocytogenes*, *Salmonella sp.* and *E. coli*). Inhibition zones were observed for the test organisms. The results indicated that all the test organisms were resistant to the *T. tetraperta* extract at concentrations ≤1%.
CHAPTER FIVE

DISCUSSION

5.1 Essential oil components (TLC and GC-MS)

The result revealed five (5) chemical compounds in the extract (Fig 1: compounds indicated with circles) using the thin-layer chromatography. In a similar study done by Aladesanmi (2006), a fractionation of the methanol extract of the fruits of *T. tetraptera* led to the isolation of a saponin glycoside with an oleanolic acid aglycone, a monodesmosidic diglycoside of the rare sapogenin 27-hydroxyolean-12 (13)-en-28-oic acid and echinocystic acid-3-0-sodium sulfate from the stem bark. In addition, umbelliferone and ferulic acid were isolated from the leaves and branches respectively. Aridanin and three of its olean-12-en-28-oic acid derivatives were also compounds that were found in the fruits by Aladesanmi (2006).

The results of the gas chromatography-mass spectrophotometry in this study further revealed thirty-two (32) compounds in the ethanol extract of the *T. tetraptera* pod. Among these were organic acids, phenols and alcohols, including eugenol. These compounds contribute greatly to the antimicrobial effects of *T. tetraptera* (Gaysinsky *et al.*, 2005). They were however, entirely different from the forty-four (44) compounds reported by Udourioh and Etokudoh (2014) (Appendix 5) whose analysis was done under the following conditions:

Oils were extracted by the hydro-distillation method using a Clevenger-type apparatus. The analysis of the oils was done using an HP6890 GC, powered by HP Chemstation Rev.A09.0 (1206) software and a flame ionization detector (FID) fitted with a fused
silica capillary column with dimension 30m x 0.25mm x 0.25μm was used. The oven temperature was programmed from 40°C -200°C at 5°C/min and run at 200°C for two minutes. Split injection temperature was 150°C with split ratio 20:1. The detector temperature was 300°C and the carrier gas was hydrogen at flow rate 1.0ml/min. Hydrogen pressure was 22psi with compressed air of 28psi.

The differences in the type of compounds observed can be attributed to the differences in the methods of extraction of the oils and the solvents used.

5.2 Sensory Analysis

There were four different treatments based on various inclusion levels of the extract (0.2%, 0.4% and 0.6%), however, the panelists did not find any one of them either more or less crumbly than the other (P>0.05) including the control (0%). The crumbliness of a product refers to how easily the product disintegrates. The result revealed similar responses for juiciness and off-flavour. There were no significant differences between the means recorded (P>0.05). However, as the inclusion levels of the extract increased, the panelists reported a significant decrease (P≤0.05) in the palatability of the products. This could be as a result of the pungent aromatic odour of the pods of *T. tetraptera* as reported by Aladesanmi (2007).

5.3 Microbial Quality

The treatment of the products with *T. tetraptera* did not significantly change microbial counts on the treated products (P>0.05) compared to the counts on the control. It is presumed that the fat composition of the products may have contributed to this observation. Studies have shown that a high fat content has the potential of reducing
the action of some essential oils as antimicrobials in meat products (Tassou et al., 1995; Gill et al., 2002). Counts were done for products that had been stored for three (3) and six (6) days under refrigeration (4 °C). No significant difference (P>0.05) was observed between the counts on the products stored for the two respective days and that of the count on the day zero (fresh) products. The observation made is possibly because the refrigeration temperature was low enough to prevent a significant growth of the microbes.

5.4 Anti-microbial Susceptibility Test

The results showed that there was no inhibition zone observed on any of the plates inoculated with the test organisms. This clearly indicated that all the test organisms were resistant to the T. tetraperta extract at the concentrations used (0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1%). This concentration range was chosen mainly because of the palatability concerns raised by the panelists when up to 0.6% of the extract was included in the formulation of the sausages. Although the last two concentrations (0.8% and 1%) were above the tolerance for palatability, they were included to test their efficacy against the three pathogens.

The resistance exhibited by the test organisms could be a result of the low concentrations of the extract (≤1%). Studies based on minimum inhibitory concentration (MIC) methods have demonstrated that herbal extracts present similar effects to most commercial antibiotics (Kamel, 2000) but need to be added in greater concentrations to demonstrate their effects (Burt, 2004). Eugenol and coriander, clove, oregano and thyme oils were found to be effective at levels of 5–20 µl/g in inhibiting
L. monocytogenes, A. hydrophila and autochthonous spoilage flora in meat products (Stecchini et al., 1993; Hao et al., 1998a, b; Tsigarida et al., 2000; Skandamis and Nychas, 2001) whilst mustard, cilantro, mint and sage oils were either less or completely ineffective in similar amounts (Shelef et al., 1984; Tassou et al., 1995; Gill et al., 2002; Lemay et al., 2002).
CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study revealed that the extract from *T. tetraptera*, whose pods are reported to be commonly used by the local people in the preparation of stews and soups, has the potential of being used in pork sausages but in acceptable amounts (up to 0.2% of inclusion) to ensure palatability, whiles benefiting from its additional nutritional value.

Three common food-borne microbes (*L. monocytogenes, S. enteric spp.* and *E. coli*) which were tested against concentrations of the extract (≤1.0%) were found to be resistant. Therefore, consumed at the levels of inclusion in the products, *T. tetraptera* is incapable of exhibiting anti-microbial effect on these pathogens.

6.2 RECOMMENDATIONS

Should *T. tetraptera* be used in the formulation of meat products, inclusion levels should not exceed 0.2% to ensure acceptable palatability.

Further studies should be conducted to determine the relative concentrations of the components of *T. tetraptera* extract and their effective inhibition doses against food-borne pathogens.

Further studies should also be done to establish the effect of *T. tetraptera* on the nutritional quality of fresh sausages.
REFERENCES


**APPENDIX**

Compounds identified by GC-MS (Udourioh and Etokudoh, 2014).

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