EXTENSION OF SHELF LIFE OF PORK USING SPICES (Plant Extracts: *Xylopia aethiopica*, *Allium sativum* and *Piper nigrum*).

BY

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CERTIFICATION

I hereby certify 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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ABSTRACT

Microbial growth, changes in colour and pH are the major problems causing shortening of shelf life in meat and meat products. Chemicals and refrigerators could be used to preserve meat. However, the chemicals are polluting to the environment and have adverse effects on human health. The refrigerators cannot be used in places where there is no electricity. The objective of this project therefore was to find natural spices with antibacterial capacities that could be potentially used as natural preservatives in fresh pork and to compare the natural spices with artificial bacteriostatic agent (vinegar). The inhibitory effects of xylopia (*Xylopia aethiopica)*, garlic (*Allium sativum*) and black pepper (*Piper nigrum*) extracts either alone or in combination and vinegar on the microbiological and chemical quality of fresh pork during storage at room temperature of 23^{o}C were investigated. Qualitative analysis of aqueous extracts of the spices tested positive for the presence of phenols, alkaloid, glycoside, terpenoids, steroids, flavonoids, tannins and reducing sugars. The results showed that addition of the extracts either alone or in combinations significantly delayed the proliferation of aerobic plate counts or extended the shelf life of the product up to nine days versus one day only for control. During storage the pH and colour parameters of extract-treated pork samples changed slightly, in comparison with significant changes in the control. Overall, the sensory scores of mixture spice-treated samples was the best, followed by the vinegar-treated meat, xylopia-treated meat, garlic-treated meat, black-pepper treated meat. The study suggests that the tested extracts, especially xylopia and combinations of the spices have potential as natural preservatives to reduce microbial growth, maintain the chemical quality and extend the shelf life of pork during storage at room temperature.

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CHAPTER ONE

1.0. INTRODUCTION

Useful shelf life is the time required for food to become unacceptable from a sensory, nutritional, microbiological or safety perspective (Labuza, 1996). The body of an animal controls microbial numbers through defensive mechanisms of white blood cells and antibody defenses. Upon exsanguination, these mechanisms are lost leading to spoilage of meat and meat products. Spoilage is a metabolic process that causes food to be undesirable or unacceptable for human consumption due to changes in sensory and nutritional characteristics (Doyle, 2007). Spoilage leads to increase in microbial load, loss of desirable colour, bad odour and flavour of meat (Faustman and Cassens, 1990). In fresh meat and meat products, microbial load indicates the safety of meat for human consumption. Also, colour is a strong indicator of quality and an important visual cue involved in consumer perception of acceptable meat quality.

Pork is a major source of food, providing a significant portion of the protein intake in the diets of a large proportion of the people, particularly in developing countries. Pork is cheap and highly acceptable, which gives it an advantage over poultry or beef (Eyo, 2001). Pork has essential sulphur-containing amino acids such as cysteine, methionine and lysine which are limiting in some legumes and most cereal-based diets (Borgstrom, 1962). In spite of the high demand of pork in Ghana, only about 50% of needs are met through local production, the rest being imported (Eyo, 2001). Pork is highly perishable, being a high-protein food with typically high levels of free amino acids which microbes metabolize, producing ammonia, biogenic amines (putrescine, histamine, and cadaverine), organic acids, ketones, and sulphur compounds (Dalgaard *et al*., 2006). Lipid degradation in fatty pork produces rancid odours and in addition, pork contains trimethylamine oxide (TMAO), the precursor of trimethylamine (TMA), the

compound responsible for pork odours generated through microbial degradation of TMAO. Prior to microbial spoilage, enzymatic and chemical deteriorative changes occur in pork because of the high content of unsaturated fatty acids and free amino acids. The high temperatures of the tropics, lack of basic infrastructures and the unsanitary production conditions prevailing in most developing countries predispose pork to spoilage. In addition to deficits in demand and supply of pork, post slaughter pork losses often reach 30 -50% in rural communities (Eyo, 2001)

In view of the demand-supply deficits in Ghanaian pork industry, it is pertinent to find ways of reducing post-slaughter losses. Apart from quantitative losses, microbial biodeterioration also causes qualitative changes in meat commodities, as most of the microbes are potential producers of toxic metabolites which are hazardous to human and animal systems (De Souza *et al*., 2005). *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enteric* are common foodborne pathogenic bacteria and are frequently isolated from various foods, including meat, milk and milk products, seafood and vegetables. The widespread occurrence of these bacteria increases the risk that foods may serve as vehicles of illness.

Growing awareness and concerns about the quality and safety of meat have led to numerous developments in meat preservation. Current meat preservation methods include heat processing, irradiation, low-temperature storage, vacuum packaging and addition of preservatives. Although none of these techniques can completely protect meat, they all play important roles. Traditionally, people around the world have used curing of meat or fish for preservation by the addition of salt, sugar, nitrite/ or nitrate. However, salt-rich diet is related to the risk of hypertension and heart disease. Nitrosamines are considered carcinogenic in animals. For this reason, nitrate is prohibited and the nitrite concentration is limited in cured meats.

These problems have led to a greater appreciation of the need for an alternative method of preservation of meat such as the use of natural preservatives. In the global food industry today, 'natural' is a powerful force as there is increasing resistance at regulatory and consumer levels against chemical food preservatives (Agatemor, 2009). Numerous naturally occurring antimicrobials are present in plant tissues and many studies have evaluated the antimicrobial activities of several plant extracts, including *Sesamum radiatum* (Shittu *et al., 2007*), *Allium cepa* (Agatemor, 2009). Dietary herbs and spices have been used for centuries for various purposes (traditional medicine, industrial applications, food preservatives and to improve sensory characteristics) because of their antimicrobial properties (Davidson and Naidu, 2000) and also most of them are categorized under GRAS (Generally Recognized as Safe) for human consumption (FDA, 2004). Bin Shan *et al.* (2008) observed that natural extracts from cinnamon stick; oregano, pomegranate peel and grape seed applied to raw chicken could reduce the final microbial load and inhibit lipid oxidation during storage at ambient temperature. Many extracts, including those from Eucalyptus, have been used to extend the shelf-life of foods, beverages and pharmaceutical and cosmetic products (Stanley, 2002). Therefore, the use of natural preservatives to extend shelf life of meat and meat products instead of synthetic preservatives is being advocated and may be of great interest to the meat industry. Perez and Anesini (1994) stressed the need to use natural plant preservatives to extend shelf-life of meat.

Xylopia (*Xylopia aethiopica*), black pepper (*Piper nigrum*) and garlic (*Allium sativum*) are among the important plants known by their fragrant odour due to essential oils in them. Although extracts of xylopia, black pepper and garlic have been reported to have medicinal and antimicrobial properties, there is little information on their inhibitory effects on foodborne pathogens and changes in colour, pH and odour in pork.

The main objective of this study, therefore, was to evaluate the ability of dietary plant extracts of xylopia, black pepper and garlic to inhibit microbial growth on pork.

The specific objectives were to:

1. Monitor the colour changes of fresh pork treated with these spices

2. Determine the changes in pH of fresh pork treated with these plant extracts.

3. Determine the effect of extracts of xylopia, black pepper and garlic on the organoleptic properties of fresh pork.

4. Evaluate the effect of muscle part on shelf life of fresh pork treated with xylopia, black pepper and garlic.

5. Determine active ingredients of xylopia, black pepper and garlic.

6. Determine the right concentrations of these extracts that can inhibit microbial growth on pork.

7. Determine the effect of the combination of spices on the fresh pork.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Pork

Pork is the culinary name for meat from the domestic pig (*Sus domesticus*). The word pork often denotes specifically the fresh meat of the pig, but can be used as an all-inclusive term which includes cured, smoked, or processed meats (ham, bacon, prosciutto, etc.). Pigs are found throughout the world especially in areas where no religious edicts prevent their rearing. They are raised for various reasons ranging from social to economics, but the ultimate purpose of rearing pigs is to provide human food in the form of fresh or processed pork to satisfy the protein needs of human beings. People use their hide for shields and shoes, their bones for tools and weapons, and their bristles for brushes. Pigs have other roles within the human economy: for example their omnivorous nature enables them to eat human rubbish, thus keeping settlements cleaner. It is one of the most-commonly consumed meats worldwide, with evidence of pig husbandry dating back to 5000 BC (Raloff, 2003).

2.2. Potentials and Constraints of Pork Production

The world trend is towards the consumption of more white than red meat. Thus the potential for increased meat production from pigs in the developing world is enormous. When compared with cattle and other ruminants, pigs have some major potential advantages including their ability to produce meat without contributing to the deterioration of the natural grazing lands (Holness, 1991). Pigs also possess the potential to be highly productive because they are capable of producing large litters after a relatively short gestation period.

Pigs have a digestive system similar to humans and different from ruminants such as cattle and sheep, which can eat forages or grasses. Pigs are fed on a diet that is primarily ground corn to supply heat and energy and soybean meal to provide protein. Vitamins and minerals are also added in their feed. Rations are closely tailored to optimize health and growth at each stage in their life. Many producers even modify the ration based on the pig's gender. It takes nearly 454 kg of feed to raise a hog to market weight. This same pig drinks about one-and-a-half to two gallons of water a day over its six-month life (Holness, 1999). These prevent small farmers from intensifying their production because the investment required often exceeds their capital wealth. Furthermore, producers are prone to production risks. These relate to resource degradation and asset control, to climatic variations such as drought and floods, and to infectious diseases. Although both small-scale and intensive livestock production systems are at risk from the predations of epidemic diseases and droughts, the poor are particularly vulnerable to these types of shocks due to their limited assets and the lack of insurance schemes (Holness, 1991). Another constraint affecting pork production is poor storage and preservation of pork leading to microbial contamination Hong *et al.* (1993) studied the effect of variable storage structures on microbial quality and shelf life of Blue Crab (*Callinectes sapidus*) meat and stated that poor storage and preservative structures increased microbial load and decreased storage life of Blue Crab.

2.2.1. Characteristics of Fresh Meat

The properties of fresh meat indicate its usefulness to the merchandiser, its appeal to the purchaser or consumer and its adaptability for further processing. Of particular importance are water-holding capacity, colour, structure, firmness and texture (Aberle and John, 2001).

Water-holding capacity (WHO) is the ability of meat to retain naturally occurring or added water during application of external forces such as cutting, heating, grinding or pressing (Aberle and John, 2001). Many of the physical properties of meat, including colour, texture and firmness of raw meat and the juiciness and tenderness of cooked meat are partially dependent on water-holding capacity (Aberle and John, 2001).

Normally, the pH of muscle drops from 7.2 (physiological) to between 5.5 and 5.8 during the immediate 24-h post-slaughter period (Brewer *et al*., 2001) of a fresh meat. This drop in pH is caused by the formation of lactic acid through anaerobic glycolysis producing denaturation and loss of solubility of proteins. In addition, the reduction of reactive groups on proteins occurs because the pH approaches the isoelectric point of myofibrillar proteins; which is the pH at which the numbers of positively and negatively charged groups tend to be attracted to each other, which limits the availability of water to bind (Aberle and John, 2001)

Changes from dark red to bright red are therefore typical and are normal reactions of fresh meat. Meat which is in the process of losing its freshness, however, no longer shows a bright red colour, even when intensively exposed to the air, because of the partial destruction of the red meat myoglobin which results in a grey, brown or greenish colour (Varnam and Sutherland, 1995).

Meat colour is mainly determined by the incident light reflectance which is dependent upon the concentration and chemical state of myoglobin pigments and the physical structure of meat. The first 30-60 minutes immediately after muscle tissue is exposed to air are critical to myoglobin oxygenation and "bloom" of muscle colour from the typical colour of reduced myoglobin (purple) to that typical oxymyoglobin (red) (Brewer *et al*., 2001). Myoglobin is the principal protein responsible for meat colour, although other haem proteins such as hemoglobin and

cytochrome C may also play a role in beef, lamb, pork and poultry colour (Harold and Hedrick, 1994).

Metmyoglobin (brown colour) formation is associated with discolouration or colour fading due to oxidation of both ferrous myoglobin derivatives to ferric iron (Harold and Hedrick, 1994). Metmyoglobin formation depends on numerous factors including oxygen, partial pressure, temperature, pH, meat reducing activity and in some cases, microbial growth (Harold and Hedrick, 1994).

Structure, firmness and texture are meat properties that are generally evaluated by consumers with visual, tactile and gustatory senses. Many factors within muscles such as rigor state and associated water-holding properties, intramuscular fat content, connective tissue content and bundle size contribute to these physical properties (Aberle and John, 2001). The texture of meat can be defined as the composite of the structural elements of meat (Varnam and Sutherland, 1995). As the meats are consumed in the cooked state, the texture of cooked meat is interpreted as tenderization (Hui and Wai-Kit, 2001). The tenderization of meat occurs in two steps; a rapid phase that is mainly due to the structural weakening of myofibrils and a slow phase caused by the structural weakening of the intramuscular connective tissue (endomysium and perimysium) (Pearson and Gillet, 1999). The freshness of meat is generally indicated by its smell. The smell of fresh meat should be slightly acidic, increasing in relation to the duration of the ripening period because of the formation of acids such as lactic acid (Hui and Wai-Kit, 2001). On the other hand, meat in decomposition generates an increasingly unpleasant odour owing to substances originating from the bacterial degradation of the meat proteins, such as sulphur compounds, mercaptane, etc (Hui and Wai-Kit, 2001).

2.3. **Microorganisms in Meat**

2.3.1. *Staphylococcus spp*

The genus *Staphylococcu*s consists of cluster-forming Gram-positive cocci. The pathogen within the genus, *Staphylococcus aureus,* causes a wide range of major and minor infections in man and animals. Currently, there are about 27 different species of *Staphylococci.* These fall into two main groups on the basis of their ability to clot blood plasma by action of the enzyme coagulase. The contamination of food can occur from either human or animal sources. Such kinds of contamination may result in staphylococcal food poisoning (Sharma and Adlakha, 1996). Staphylococcal food poisoning is the name of the condition caused by the enterotoxins which some strains of *S. aureus* produce.

2.3.2. *Pseudomonas spp*

Pseudomonas spp which are aerobic, are among the most common spoilage agents of meat and meat products (Cross and Overby, 1998). *Pseudomonas spp* is Gram-negative psychrotrophs, which grow, although slowly at refrigeration temperatures (below 7 ^{o} C) but optimally at temperatures above refrigeration, for example, 25-30 ^{o} C. Their maximum temperatures are 30-35 ^{o}C. Growth of *Pseudomonas spp.,* like that of other Gram-negative psychrotrophs, is affected by oxygen tension, salt and other food additives, water activity (a_w), pH and other factors. During growth, *Pseudomonas* produces protease and lipases that can catalyze reactions causing degradation of protein and fat. The consequence of these reactions is formation of peptides and fatty acids of undesirable flavour (bitterness, rancidity) and odour. Sometimes these bacteria also produce unsightly green pigments (Sharma and Adlakha, 1996). The genus *Pseudomonas*

comprises more than 200 species, mostly saprophytes found widely in soil, water and other moist environments.

2.3.3. *Enterobacteriaceae*

The *enterobacteriaceae* are a large heterogenous group of Gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (*Escherichia, Shigella, Salmonella, Enterobacter, Klelosiella, Serratia* and *Proteus*). The *Enterobacteriaceae* are facultative anaerobes or aerobes which ferment a wide range of carbohydrates, possess a complex antigenic structure and produce a variety of toxins and other virulence factors (Sharma and Adlakha, 1996). The *Enterobacteriaceae* are useful indicators of hygiene and post-processing contamination of processed foods. Their presence in high numbers in foods indicates that an unacceptable level of contamination has occurred or there has been under processing (e.g. inadequate cooking) (Hui *et al,* 1994).

2.3.4. *Coliforms*

Coliforms are general term for facultative Gram-negative rods that inhabit the intestinal tracts of humans and animals without usually causing diseases. Sharma and Adlakha (1996) stated that fecal coliforms are bacteria that ferment lactose to produce acid and gas at 44.5 ^{o}C up to 48 hours and hence give bad odour. They include the genera *Escherichia, Enterobacter,* and *Klebsiella* and they are used as indicator organisms in water quality testing. They are a common indicator of faecal contamination and a possible presence of other pathogens (Sharma and Adlakha, 1996). In general, increased levels provide a warning of failure in water treatment, a

break in the integrity of the processing and possible contamination with pathogens. When the levels are high, there may be an elevated risk of gastroenteritis.

2.3.5. Factors Influencing the Microbial Growth on Meat and Meat Products

Though meat handling, storage and consumption may differ from one place to another, the factors limiting the shelf-life of these products are the same. These factors may be classified into endogenous and exogenous.

The endogenous factors include:

- Nutrient content
- Antimicrobial constituents
- pH-value or the degree of acidity of the product
- a_w value or the amount of moisture available in the product and

The exogenous factors include:

- oxygen (from the air);
- micro-organisms;
- temperature;
- light; and
- evaporation and desiccation.

Source: Cross and Overby, (1998).

2.3.5.1. Nutrient Content

Microorganisms require basic nutrients for their growth and the maintenance of metabolic functions. These nutrients include water, nitrogen, vitamins and minerals. The content of these nutrients varies from food to food. Meats have abundant protein, lipids, minerals and vitamins but low levels of carbohydrates. Foods, such as milk and milk products and eggs, are rich in nutrients. Many microorganisms derive their energy from carbohydrates, alcohols and amino acids. They can metabolize simple sugars such as glucose. Other bacteria can metabolize more complex carbohydrates such as starch or glycogen found in meat and meat products (Cross and Overby, 1998). Some microorganisms are able to metabolize peptides and more complex protein to gain amino acids which serve as a source of nitrogen and energy. Other sources of nitrogen include, for example, urea, ammonia and creatinine. Fats are rarely used as a source of energy (Cross and Overby, 1998).

2.3.5.2. Antimicrobial Constituents

Some foods contain antimicrobial compounds, for example, eugenol in cloves, allicin in garlic, lactofferin, conglutinin and the lactoperoxidase system in cow's milk, lysozyme in eggs and milk and other compounds in fresh meat, poultry and seafood. Gram negative psychrotrophs, such as, the Pseudomonasdaceae have been shown to be very sensitive to the lactoperoxidase system (Cross and Overby, 1998).

2.3.5.3. Combined Effect of pH and A_w -Value

Generally, the longer the shelf-life of meat and meat products will be the lower the pH-value and/or a_w-value. Both factors (either pH or a_w alone or the two together) have a decisive

influence on the growth of micro-organisms in food (Cross and Overby, 1998). However, there are limits for most meat products regarding decreased pH-value and a_w-value, particularly for organoleptic reasons.

2.3.5.4. Sources of Microbial Contamination

The primary sources of microbial contamination on meat are soil, air, animal feed and animal hides (Bell, 1997). Microbial status of fresh meat depends on animal transportation, slaughtering and cutting and packaging. If these processes are not done under hygienic conditions, they could lead to microbial contamination of meat (Biswas *et al.*, 2011). Hides, hooves and hair contain not only large numbers of microorganisms from soil, manure, feed and water but also important kinds of spoilage organisms (Biswas *et al.*, 2011). The faeces and faecal contaminated products can contain many enteric organisms including Salmonella. Most contamination of faecal origin occurs during hide or skin removal and evisceration processes in the slaughterhouse (Sofos *et al.*, 1999). People working in meat processing plants also serve as vector of many pathogenic bacteria (Zweifel *et al.*, 2008). Gill (1998) reported that potential meat contamination of *Streptococcus faecalis* occurred during slaughtering and butchering of food animals. He further revealed that knife trimming do not contribute to enhancing microbiological quality of dressed carcasses, except aesthetic values.

2.4.0. Assessing Microbial Numbers, Growth and Activity in Meat

Aerobic Plate Count (APC) is a technique used to determine the number of microorganisms in food product or an ingredient (Cousin *et al.*, 1992). These data are often used as indicators of food hygienic quality or predictors for the shelf life of a product. The media used for APC-

determination do not contain any selective or differential additives. The colonies counted after inoculation and incubation of the sample aliquots only represent the organisms that could multiply at the given conditions of growth (temperature, incubation period, media and atmosphere). The number of colonies that develop after an incubation period gives an indication of total microbial numbers. Other populations of organisms that can only grow at higher or lower temperatures or that grow very slowly or require additional nutritional components or need a specialized atmosphere such as a reduction in oxygen or decrease in carbon dioxide or nitrogen will not be part of the APC. Not much information on the type of organisms is given by the APC technique.

2.4.1. Control of Microbial Contamination

2.4.2. Sanitation

According to FAO (1999), microbial contamination of meat could be controlled by following strict sanitation procedures. Personal hygiene by washing of hands with detergents and sterilization of all equipments and working surfaces are important to control microbial contamination of meat (FAO, 1999). Microbial contamination of meat could also be contolled by controlling pests which serve as vectors of many pathogenic organisms (FAO, 1999).

Jordan *et al* (1999) reported that vertical integrated approach to food safety (farm-to-table) where control is exerted at all stages of meat production is ideal to control microbial contamination of meat. This approach would include control in farm/herd management, transport and handling controls between farm and slaughter, pre-slaughter and slaughter controls, product processing controls, controls in handling and transport of products, and proper cooking and handling by consumers.

Quality control Systems such as pre-operational, raw material, process and final product monitoring are very vital in controlling microbial contamination of meat and meat products FAO, 1999. In a recent comment, it is explained Food and Agricultural Organization or World Health Organization (FAO/WHO) need to do a much better job on regulatory enforcement and they need better enforcement tools too (Haque *et al.*, 2008). So, if one country is willing to export meat and meat products to other country, there is a need for regular monitoring of meat and meat products according to international standards or guidelines set by that country. Meat importing countries should also maintain strict inspection and sampling programmes for vigilance against accidental or international contamination (Haque *et al.*, 2008).

2.4.3. Packaging

Packaging plays an important role in providing desired characteristics of meat to consumers. Initial purchase decision is based on the appearance of the product (Ralston 1985). According to Kerry *et al* (2002), the colour of uncooked meat and meat products is usually described as pink or red but it ranges from nearly white to dark red. Discolourations of these products often involve tan, brown, gray, green or yellow (Pearson and Gillet, 1999). The use of packaging in the meat industry provide containment of the meat or raw materials, protection against physical and chemical changes, protection against contamination by either microbial, physical or chemical agents, provision of attractive appearance for sale, convenience and use. Packaging is therefore essential for maintaining the product's quality and identity (Aberle and John, 2001).

2.5.0. Meat Preservation

2.5.1. Dried Meats

Drying meat under natural temperatures, humidity and circulation of the air, including direct influence of sun rays, is the oldest method of meat preservation. Preservation results from the removal of water initially from the surface and later throughout the meat, reducing the water activity to a level where microorganisms are unable to proliferate (Pearson and Gillet, 1999). Continuous evaporation and weight losses during drying cause changes in the shape of the meat through shrinkage of the muscle and connective tissue. The meat pieces become smaller, thinner and to some degree wrinkled (FAO, 1999). The consistency also changes from soft to firm to hard. In addition to these physical changes, there are also certain specific biochemical reactions with a strong impact on the organoleptic characteristics of the product. For that reason, the specific flavour of dried meat is completely different from the characteristic flavour of fresh meat. Slight oxidation of the meat fats contributes to the typical flavour of dried meat. High temperatures during meat drying and storage cause intensive oxidation (rancidity) of the fat and an unpleasant rancid flavour which strongly influences the palatability of the product (FAO, 1999).

2.5.2. Frozen meat

Because microbial growth is inhibited at temperatures below approximately -3 to -5 ^{o} C, freezing is an excellent method for the preservation of meat and meat products to protect against microbial public health hazards and spoilage. The freezing process and in particular, frozen storage gradually destroy microorganisms such as gram-negative bacteria and *Clostridium*

perfringens. *Salmonella* and the gram-positive microflora are affected less by freezing and the surviving bacteria will be able to multiply when suitable temperatures are reached during or after thawing. It is recommended that frozen meat be thawed at refrigeration temperature rather than at room temperature, because in the latter case the surface temperature may be conducive to bacterial growth, while the center is still frozen (FAO, 1999).

2.5.3. Cooked Meat

Cooked meats are produced in a number of different ways. Some meat products may be heated to pasteurization temperatures, others to well above 100 ^{o}C. Some cooked meats are sold to the consumer wrapped in paper or plastic, while others are packaged in hermetically sealed containers. The heat resistance of the microorganisms that will grow in meat and meat products varies considerably. Heat treatment may not necessarily kill bacteria, but will inactivate them so that after prolonged exposure to optimal temperatures they will then recover and multiply. The action of other preservation methods, such as the addition of salt and nitrite, is to effectively prolong the time necessary for recovery of heat-exposed bacteria. Post-processing contamination of cans of corned beef and pork products have caused incidents of staphylococcal food poisoning (Stephen and Morgan, 1995).

2.5.4. Smoked Meat

Smoking of meat is a technique in which meat is exposed directly to wood smoke which may be generated by a variety of methods. In smoke produced from wood, there are various substances which contribute to the flavour and the appearance of the smoked meat product and which have a certain preserving effect on the product. However, the preserving effect of common smoking is

not very significant when storing the product without a cold chain. On the other hand, intensive or prolonged smoking may considerably increase the shelf-life of the product, but it also has an unfavourable effect on flavour. The smoke from most wood is also known to be carcinogenic in animals and human beings. In view of the above, smoking in order to preserve meat can only be considered as an emergency measure when no other preservation methods can be carried out. This may be the case during wet weather or generally under a humid climate, or when the preservation has to be completed as fast as possible because of the need of immediate transport, for instance after game-hunting (FAO, 1999).

2.5.5. Use of Antimicrobial Agents

2.5.5.1. Common Salt (Sodium Chloride)

It has been an ancient practice to incorporate salt into meat to increase shelf-life and enhance flavour. Common salt is an essential ingredient in meat processing. It serves as a preservative, flavouring agent and as solubilizer of proteins. Salt migrates into the muscle fibers and result in swelling of the fibers and thus play an important role in the solubilization of the myofibrillar proteins, which contribute immensely to the texture of the meat (Kerry *et al*, 2002). The preservative effect of salt is primarily the result of the muscle tissue having a higher concentration of salt than the bacterial cells. Most bacterial cell walls are semi-permeable in nature and will permit water but not salt to pass through them. Water will then pass from the less dense to the more dense concentration to result in shriveling of bacterial cell wall. Too much salt is, however, reported to cause hypertension (Kerry *et al*, 2002).

2.5.5.2. Spices (natural plant extracts).

"Spice" is a culinary term, not a botanical category. It does not refer to a specific kind of plant or plant part (Farrell, 1990). Indeed, spices come from various woody shrubs and vines, trees, aromatic lichens, and the roots, flowers, seeds, and fruits of herbaceous plants. Cookbooks generally distinguish between seasonings (spices used in food preparation) and condiments (spices added after food is served), but not between herbs and spices. However, herbs, which are defined botanically as plants that do not develop woody, persistent tissue, usually are called for in their fresh state, whereas spices generally are dried. Salt is sometimes thought of as a spice, but it is a mineral. Spices are plant products used in flavouring foods and beverages. For thousands of years, aromatic plant materials 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; Dillon and Board, 1994).

During and after the middle ages, seafarers such as Marco Polo, Ferdinand Magellan, and Christopher Columbus undertook hazardous voyages to establish routes to trading ports in primary spice-growing regions (Parry, 1953). The spice trade was so crucial to national economies that rulers repeatedly mounted costly expeditions to raid spice-growing countries, and struggles for the control of these countries precipitated several wars. When Alarich, a leader of the Goths, laid siege to Rome in AD 408, he demanded as ransom various precious metals and 3000 pounds (metric) of pepper (Scheiper, 1993).

Each spice has a unique aroma and flavour, which are derived from compounds known as phytochemicals or "secondary compounds" because they are secondary to the plant's basic metabolism.

2.5.5.2.1. Antimicrobial properties of spices

The obvious answer to why spices are used is that they enhance food flavour, colour, and palatability. Of course, this is true as far as it goes. However, such a proximate (immediate cause) explanation does not address the ultimate (evolutionary) questions of why cuisines that contain pungent plant products appeal to people and why some phytochemicals are tastier than others. Answers to proximate and ultimate questions are complementary, not mutually exclusive, and full understanding requires explanations at both "levels of analysis" (Sherman, 1988). A clue to the ultimate reason for spice use may lie in the protective effects of phytochemicals against plants' biotic enemies. These chemicals evolved in plants to protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites (Fraenkel, 1959; Walker, 1994). Most spices contain dozens of secondary compounds. These are the plants' recipes for survival-legacies of their coevolutionary races against biotic enemies. Some animals that store food add plants with antibacterial and antifungal properties to their caches (e.g., brown bears sometimes cover carcasses with Spaghnum moss (Elgmork, 1982).

After all, meat and other food items are also attacked by bacteria and fungi, indeed by some of the same species that afflict plants. Throughout recorded history, foodborne bacteria (especially species of *Clostridium, Escherichia, Listeria, Salmonella, Shigella,* and *Vibrio*) or their toxins have been serious health concerns, and they still are (Hui *et al.*, 1994; WHO, 1999). If spices were to kill such microorganisms or inhibit their growth before they could produce toxins, use of spices might reduce foodborne illnesses and food poisoning (Billing and Sherman, 1998). Microbiologists and food-product developers have conducted laboratory experiments that involve challenging numerous foodborne bacteria, fungi, and yeasts with phytochemicals extracted from spice plants. Multiple techniques have been used to investigate inhibition, and the

primary data vary considerably in quality and quantity for different spices. Nevertheless, it is now clear that many spices have potent antimicrobial properties (Hargreaves *et al.,* 1975; Shelef 1984; Deans and Ritchie, 1987; Zaika, 1988; Beuchat, 1994, Nakatani, 1994; Hirasa and Takemasa, 1998)

Use of spices should be of the greatest benefit in hot climates, where unrefrigerated foods easily spoil. Uncooked meats and meat dishes that are prepared in advance and stored at room temperatures for more than a few hours typically build up massive bacterial populations, especially in tropical climates (Hobbs and Roberts, 1993). Unrefrigerated meats spoil faster than vegetables and are more often associated with foodborne disease outbreaks (Sockett, 1995) suggesting that vegetables (plant tissues) contain more antimicrobial compounds.

Regarding the effects of cooking, most phytochemicals are thermostable, although a few are destroyed by heat (Moyler, 1994). Some spices (for example, garlic, pepper, rosemary, and onion) are typically added at the beginning of cooking, whereas others such as parsley and cilantro, i.e coriander, leaf are added near the end. According to cookbook authors, the "delicate" flavors of the latter would be destroyed by heat. If, as seems likely, thermostable spices are the ones added early and thermostable spices are added later (or are used primarily as condiments). Differences in timing of use may function to maintain beneficial antimicrobial properties until food is served.

2.5.5.2.2 Spice synergism

Pepper, lemon and lime juice are among the most frequently used spices, but they are unusual in that the frequency with which they are used does not change much across the temperature gradient. Pepper and citric acid play special roles, that is, as synergists. Citric acid potentiates the

antibacterial effects of other spices because low pH disrupts bacterial cell membranes (Booth and Kroll, 1989). Foods to which lemon or lime juice are added require less heating to cause the same levels of bacterial mortality that take place in foods cooked at higher pH and temperature for a longer time. Black pepper comes from *Piper nigrum*, an exclusively tropical plant that has several useful properties. For example, the compound piperine inhibits the ubiquitous, deadly bacterium *Clostridium botulinum* (Nakatani, 1994). Black pepper is also a "bioavailability enhancer," meaning that it acts synergistically to increase the rate at which cells, including microorganisms, absorb phytotoxins (Johri and Zutshi, 1992). Many other spices exhibit greater antibacterial potency when they are mixed than when used alone (Ziauddin *et al*,. 1996). Some are combined so frequently that the blends have acquired special names. An intriguing example is the French "quatre epices" (pepper, cloves, ginger, and nutmeg), which is often used to make sausages. Sausages are rich medium for bacterial growth and have frequently been implicated as the source of botulinum toxin. Other blends, such as curry powder (which contains 22 different spices), pickling spice (15 spices), and chili powder (10 spices), are broadspectrum antimicrobial melanges

In addition to their uses in cooking, individual spices and blends are employed as colouring agents, antivirals (including suppressing HIV), brain stimulants, and aphrodisiacs (Hirasa and Takemasa, 1998).Among traditional societies, many spice plants also have ethnopharmacological uses, often as topical or ingested antibacterials and vermicides (Chevallier, 1996; Cichewicz and Thorpe, 1996). A few spices, particularly garlic, ginger, cinnamon, and chilis, have for centuries been used to counteract a broad spectrum of ailments, including dysentery, kidney stones, arthritis, and high blood pressure (Johns, 199; Duke, 1994).

2.5.5.2.3 Antioxidant activity of spices

Spices have achieved some commercial importance as antioxidants. Beneficial influence of certain ground herbs and spices in fat stability has been known (Schuler, 1990). A systematic investigation during four years and covering 32 species of herbs and spices has been carried out by Chipault *et al.,* 1952. Several food models have been tested under various conditions including lard: active oxygen method (AOM); emulsion: Warburg apparatus; ground pork: frozen storage; mayonnaise: storage at room temperature; pie crust: storage at 63 ^{o}C and the peroxide values determined. The antioxidant efficacy was expressed as a protection factor indicating the ratio of carbon dioxide absorption in food model without spice. This is a measure of stability. The effectiveness of spices and herbs depend not only on variety and quality but also on substrate and storage conditions (Schuler, 1990). The antioxidant compounds of spices have been investigated. In rosemary, carnosic acid has been described as the most active antioxidant constituents by Brieskorrn and Domling, (1969). Other phenolic compounds have been investigated, for example, rosemaric acid, which has in model system in activity comparable to that of caffeic acid. Antioxidant extract from spices (usually rosemary) commercially available are in fine powderd form. Depending on their content of active substances, it is recommended they should be used at levels between 200 and 1000 mg/kg of finished product to be stabilized (Schuler, 1990). Generally, the powders are dispersible in oils or fats, insoluble in water, but soluble in organic solvents. Due to their powder characteristics, they can also be used in the dry mixes. The antioxidant activities of crude extract and three fractions along with 13 subfractions obtained from the ethyl acetate (EA) soluble fractions of ethalp-nolic extract of the bark of *Chamacyparis obtuse* var *formosana* were assayed for several antioxidant (Palanisamy et al., 2008). The EA soluble fraction was found to be the best antioxidant-rich fraction in terms of

DPPH and reducing power assays. With further analysis it was found that there was a positive correlation between the total phenolic content of extracts and Trolox equivalent antioxidant capacity (TEAC). It was concluded that the EA fractions of *Chamacyparis obtuse* var *formosana* bark extract showed a strong radical scavenging and can be considered a good source of natural antioxidants for medicinal and commercial use. Bin Shan *et al.,* 2009) observed that clove was the most effective for retarding lipid oxidation and presented the highest antioxidant activity in meat. The spice and herb extracts contained high levels of phenolic compounds which contributed to lower pH values in meat and were involved in the maintenance of meat colour through their antioxidant effects. As the final pH increased, the meat gradually became darker. High final pH also affected the colour stability of the raw meat because it affected enzyme activity and the rate of oxygenation. Reducing enzymes are necessary to convert metmyoglobin back to oxymyoglobin which is much known to contribute to the colour of fresh meat (Pietrasik *et al*., 2006).

Flavour agents are added to pork to provide alternate flavour choices for consumers by incorporating fresh ingredients, dehydrated ingredients, ground spices, spice extractives or oleoresins into the final product (Miller, 1998). In addition, flavour agents can also be used to mask undesirable flavours produced from other ingredients. While being acceptable for the consumer, the addition of whole spices can decrease the microbial load either through addition of antimicrobial active ingredients contained within the spice or by the inherent antioxidation potential contained within the ingredient (Miller, 1998). Many spices are treated either by irradiation or by chemical decontamination methods to reduce the microbial population in the spice so that their addition does not affect shelf–life. The oxidation potential of fresh ingredients can only be prevented by processing the ingredients to disenable the oxidation potential. On the

other hand, some spices and ingredients (rosemary, garlic, and onions) have antioxidant properties that improve flavour stability during storage.

2.5.5.2.4. Costs of spices

In light of the beneficial effects of spices, questions are asked why are spices not used everywhere. The answer probably lies in the costs of spice used, including financial costs to procure parts of plants that do not grow locally (for example, consider the price of Spanish saffron), illnesses caused by ingesting spices that are themselves contaminated (for exsample, with bacteria, fungi, or animal feces), and other hazards of ingesting too many plant secondary compounds and essential oils. Indeed, Ames *et al*., 1990 and Beier and Nigg, (1994) reviewed evidence that phytochemical in many common spices have mutagenic, teratogenic, carcinogenic, or allergenic properties. For example, small quantities of chilis have been found to have antimicrobial and therapeutic effects, but ingestion of large amounts of capsaicin have been associated with necrosis, ulceration, and carcinogenesis (Surh and Lee, 1996). The implication is that too much of a good thing can have negative effect.

In hot climates, benefits of avoiding food borne illnesses and food poisoning apparently outweigh the various costs of spices. In countries where spices are heavily used, pre-adolescent children and women in their first trimester of pregnancy (Profet, 1992) typically avoid highly spiced foods, especially meats. These differences in spice use may have a similar adaptive basis. For example, Profet, 1992 suggested that morning sickness may function to reduce maternal intake of foods containing teratogens during the early phase of embryogenesis, when delicate fetal tissues are most susceptible to chemical disruption. Indeed, women who experience morning sickness are less likely to miscarry than women who do not (Weigel and Weigel, 1989).

Young children, who are growing rapidly, may also be particularly sensitive to environmental mutagens. Once pregnancy has progressed into the second trimester and once children reach puberty, the dangers of food poisoning and food borne illnesses may again outweigh the mutagenic risks associated with phytochemicals (Flaxman and Sherman, 2001). Interestingly, maternal ingestion of spices late in pregnancy or during lactation can slightly bias offspring toward accepting spices (Altbacker *et al*., 1995).

2.5.6.0. Organoleptic Evaluation of Meat Quality

Organoleptic evaluation consists in describing the attributes of food, in this special case, of meat and meat products, which can be perceived by the sense organs. The attributes to be evaluated are appearance, colour, texture and consistency, smell and taste.

2.5.6.1. Appearance

The appearance of meat, either as a carcass or as boneless meat cuts, has an important impact on its objective or subjective evaluation. Although in modern grading procedures, more and more technical equipment has been incorporated, visual methods are still in use. They can be of special value in most developing countries where no extremely sophisticated methods are needed. The way the consumers or the processors check the appearance of meat is subjective. Differences will be registered in the relation of lean meat and fat including the degree of marbling or in the relation of bones and lean meat. Furthermore, unfavourable influences can be detected such as unclean meat surfaces, surfaces too wet or too dry, or unattractive blood splashes on muscle tissue. Special product treatments (for instance chilling, freezing, cooking, curing, smoking,

drying) or the kind and quality of portioning and packaging (casings, plastic bags, and cans) will be recognized by evaluating the appearance (FAO, 1999).

2.5.6.2. Colour

Under normal circumstances, the colour of meat is in the range of red and may differ from dark red, bright red to slightly red; but also pink, grey and brown colours may occur. In many cases, the colour indicates the type and stage of the treatment to which the meat has been subjected, as well as the stage of freshness.

In judging meat colour, some experience is needed to be able to distinguish between the colour which is typical for a specific treatment or which is typical for specific freshness. Furthermore, meat derived from different species of animals may have rather different colours, as can easily be seen when comparing beef, pork and poultry meat (FAO, 1999). Remarkable changes in the meat colour occur when fresh meat has been boiled or cooked. It loses its red colour almost entirely and turns to grey or brown (FAO, 1999). The reason for this is the destruction of the myoglobin through heat treatment. This is the typical colour shown in sausages of all types, raw and cooked hams, corned beef, etc (FAO, 1999). Cured products with a decreasing keeping quality can be recognized when the red colour becomes pale or changes to grey or green.

2.5.6.3. Texture and consistency (tenderness and juiciness)

Meat prepared for the consumer should be tender and juicy. Meat tenderness depends on the animal species from which the meat originates. Lamb, pork and poultry meat are sufficiently tender after slaughter, but beef requires a certain period of maturation to achieve optimal eating quality (FAO, 1999).

Texture and consistency, including juiciness, are an important criterion, still neglected by many consumers, for the eating quality of meat. The meat should be cooked to become sufficiently tender, but cooking should not be too intense otherwise the meat becomes dry, hard and with no juiciness (FAO, 1999). The simple way to check the consistency of foods is by chewing. Although this test seems easy, in practice it is rather complicated. Taste panelists need experience, particularly when the different samples have to be ranked, for example, which sample is the toughest, the second toughest or the most tender. On the other hand, inappropriate processing methods (too intensive cooking, curing, comminuting) may cause losses in the desired consistency and juiciness, and the best way to check this is by chewing (FAO, 1999).

2.5.6.4. Smell and taste (aroma and flavour)

The flavour of fresh meat can also be checked by putting small samples (approx. 10 pieces of 1 cm^3 each) in preheated water of 80°C for about five minutes (boiling test). The odour of the cooking broth and the taste of the warm meat samples will indicate whether the meat was fresh or in deterioration or subject to undesired influences (FAO, 1999). When processing the meat, the smell and taste of the meat products can differ a great deal owing to heat treatment and the use of salt, spices and food additives. Every meat product has its typical smell and taste, and the test person should know about it (FAO, 1999). Panelists should not smoke or eat spicy meals before starting the test and should rinse their mouth frequently with water during the test (FAO,

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Types and Sources of Plant Materials Used in the Study

The three dietary plant materials (spices) used in the present were xylopia (*Xylopia aethiopica* ((Dunal) A. Rich,), garlic (*Allium sativum* L.) and black pepper (*Piper nigrum*). The pictures of xylopia, garlic and black pepper are shown in Plate 3.1, 3.2 and 3.3, respectively.

The dry bulbs (Garlic) and dry fruits (Xylopia and Black pepper) were purchased from local markets in Kumasi, Ghana. Hulls were manually separated from the spices and the spices were milled prior to extraction. The scientific name and origin of the three spices are presented in Table 3.1.

Plate 3.1. Fruits from *Xylopia aethiopica* still attached to the tree (left), and a close up of the sun dried fruit (right).

Plate 3.2. Bulbs of garlic.

Plate 3.3. Leaf and fruits of black pepper.

Table 3.1. Scientific, common name and Origins of *Xylopia aethiopica, Allium sativum* and *Piper nigrum*.

Spice	Family	Common name	Origin	Plant part used
Xylopia aethiopica	Annonaceae	Negro pepper, Guinean pepper Local name (Ghana)-Hwentia		Fruit
Allium sativum	Liliaceae	Garlic	Southwest of Siberia	Bulb
Piper nigrum	Piperaceae	Black pepper	Brazil, India, Sri Lanka, Indonesia, Malaysia	Fruit (berries)

SOURCE: Belitz and Grosch (1987)

3.2. Preparation of Plant Extracts

Plant sample (garlic) was cleaned, freeze-dried and ground to a fine powder (71µm) in a Kenwood-Mill (Kenwood Ltd, Havant, UK) and passed through a 24-mesh sieve. Dried plant samples (Xylopia and black pepper) were also cleaned and ground to a fine powder and passed through the sieve as described above. 20g, 30g, and 50g of *Xylopia aethiopica*, *Allium sativum*, *Piper nigrum* respectively and a mixture (equal proportions of the three spices) were added to 500 ml of hot sterile distilled water. The mixtures were vigorously stirred and were left to stand for 1 h at room temperature (25 ^{o}C). The extract was filtered through a single sheet of Whatman No. 1 filter paper by gravity. The extracts were stored at 4 ^{o}C until use.

3.3. Phytochemical Analysis of Plant Extracts

Chemical analysis were conducted to determine the presence of the active constituents such as phenols, alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin using the following procedures:

3.3.1. Phenols/polyphenols

Four grams of the material was extracted in ethanol and evaporated to dryness. Residue was dissolved in 20 ml distilled water and 0.5 ml Folin-ciocalteau reagent was added followed by 2 ml of 20% Na_2CO_3 solution. Development of bluish colour indicated the presence of phenols (Sadasivam and Manickam, 1996).

3.3.2. Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Meyer's

reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Meyer's reagent (Evans, 2002). The alcoholic extract of the material was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

3.3.3. Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added and observed for a reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer (Siddiqui and Ali, 1997).

3.3.4. Terpenoids and Steriod

Four milliliters of the extract solution was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Concentrated solution of sulphuric acid was then added slowly. The appearance of red violet colour indicated the presence of terpenoid and green bluish colour for steroids (Siddiqui and Ali, 1997).

3.3.5. Flavonoids

Four milliliters of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution 5-6 drops of

concentrated hydrochloric acid was added. The appearance of red colour indicated the presence of flavonoids and orange colour for flavones (Siddiqui and Ali, 1997).

3.3.6. Tannins

To 0.5 ml of extract solution 1ml of water and 1-2 drops of ferric chloride solution were added. The appearance of blue colour indicated the presence of gallic tannins and green black for catecholic tannins (Iyengar, 1995).

3.3.7. Reducing Sugar(s)

To 0.5 ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution were added to show the appearance of brick red precipitate.

3.4. Preparation of Meat Samples

Fresh pork samples were purchased from the Kumasi Abattoir and immediately transported in a cooler containing crushed ice to the Microbiology laboratory, Department of Biological Science, KNUST and prepared for testing on the day of purchase. The fresh pork was obtained from loin, shoulder and topside muscles. These muscles were utilized for the study. Excess fat and accessory muscles on the surface were trimmed with a sanitized knife under aseptic conditions. The meat from each muscle was cut into pieces of uniform weight (20g) for microbiological analysis. Fresh pork from loin, shoulder and topside muscles was divided into fourteen batches (twenty pieces (20g) per batch). Each batch was dipped for 30 min in aqueous solution formulated to contain either Xylopia (20g/500ml), Xylopia (30g/500ml), Xylopia (50g/500ml), Garlic (20g/500ml), Garlic (30g/500ml), Garlic (50g/500ml), Black pepper (20g/500ml), Black

pepper (30g/500ml), Black pepper (50g/500ml), Combination of Xylopia+ Garlic + Black pepper (6.7g+ 6.7g+ 6.7g/500ml), Combination of Xylopia+ Garlic + Black pepper (10g+ 10g+ 10g/500ml), Combination of Xylopia+ Garlic + Black pepper (16.7g+ 16.7g+ 16.7g/500ml), Vinegar (Acetic acid) or no antimicrobial agent (control) and gently swirled with a sterile glass rod. All treatment solutions were maintained at room temperature (25 ^{o} C). The pieces of pork were then removed from the solution and allowed to drain on a stainless wire mesh screen for 10 min. Subsequently, the treated samples were individually placed in sterile plastic containers, labeled and stored at room temperature. Pieces of pork from the fourteen groups were sampled periodically at storage days 0, 1, 3, 5, 7 and 9 for pH, visual colour, and microbial load.

3.5. Microbiological Analyses

Meat samples of 20g were removed from the plastic containers and homogenized in 200 ml of sterile distilled water (water was autoclaved). From this homogenate, decimal serial dilutions were made in the same sterile distilled water and used for microbiological analyses of the pork samples at each of the appropriate time intervals during storage. Serial dilutions for inoculation were prepared according to the procedure outlined by ICMSF (1986). A test tube was filled with 10 ml of the solution (homogenate). The rest of the test tubes were filled with 9 ml of the buffer (sterile distilled water) for serial dilution of the original solution.

Aerobic plate counts (APC) were determined by inoculating 0.1 ml of the sample homogenate, at selected dilutions, onto triplicate sterile plates of pre-poured Agar using the surface spread technique, and then the plates were incubated for 48 h at 35°C. Microbial load on the pork samples were determined by counting colony forming units (CFU) that developed on the plates after incubation as shown in Plate 3.4.

Plate 3.4. The researcher counting the CFU.

3.6. pH Measurement

Twenty grams of samples of each muscle from each treatment were homogenized in 50 ml distilled water. The homogenate was filtered and the pH of the filtrate was determined with a standardized combination electrode attached to an ATI Orion 720A benchtop pH meter (Orion Reseearch, Inc., Boston, MA, USA) as shown in Plate 3.5. Three readings were taken from each sample.

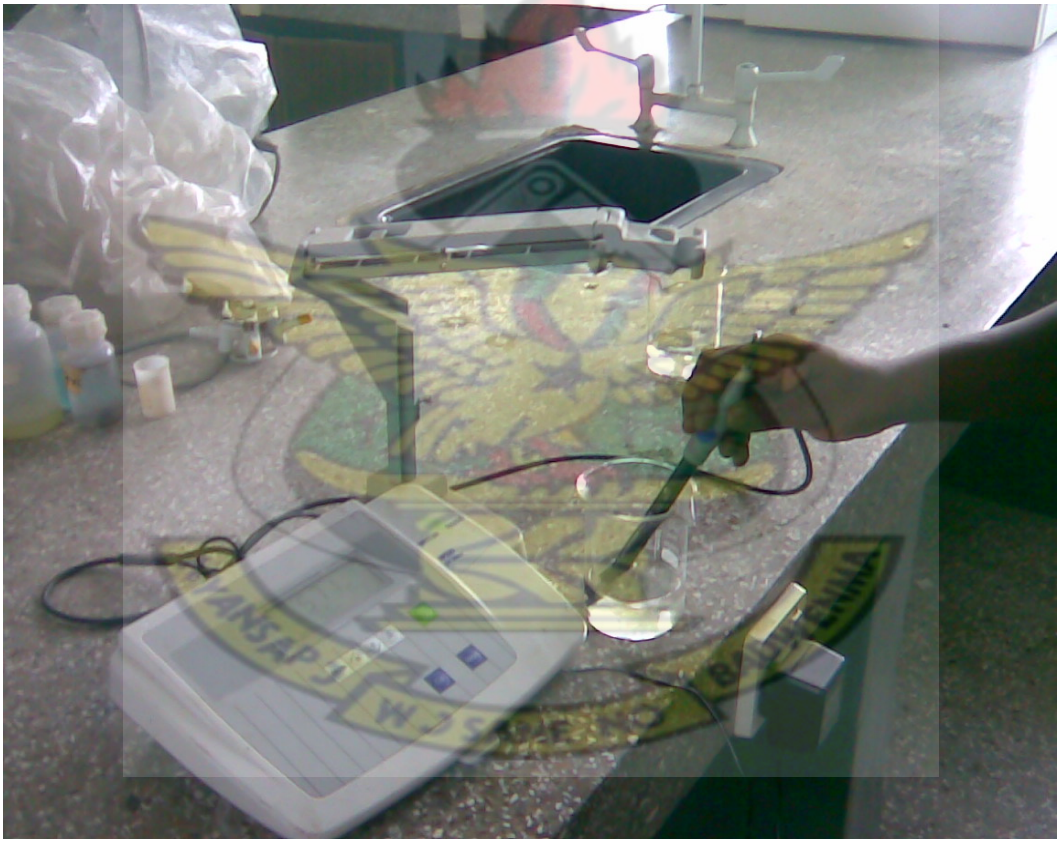


Plate 3.5. The electrode of bench top pH meter dipped into the homogenate.

3.7. Colour Observation

Colour changes occurring on the surface of meat samples during storage were monitored by visual observation.

3.8. Sensory Evaluation

Sensory evaluation was performed to investigate the acceptance of cooked meat prepared from the treated samples. A trained panel consisted of 10 students (mean age 22.9 years) and other information on the panel like whether they have taken pork before was collected from a questionnaire. The samples were cut into cubes of uniform size, cooked in a pressure cooker at 15 pounds pressure for 5 minutes and served warm with code numbers to the trained 10 member consumer panel. Water was provided for mouth rinsing between consecutive samples. A standard nine- point category scale was used for evaluation of acceptance of samples (1= strongly dislike, 9= strongly like).

3.9. Statistical Analysis:

All data are presented as means and all measurements were carried out in triplicates. All microbial counts were converted into base-10 logarithms of colony forming units per g of pork samples (log CFU g). Data were subjected to analysis of variance (ANOVA) to determine the differences in the different treatments. Significant differences among the means were determined by Least Significant Difference (LSD) test. All data analysis was performed using the Genstat statistical package (Version 5).

CHAPTER FOUR

4.0 RESULTS

4.1. Phytochemical Screening of Spices

Qualitative analysis of aqueous extracts of xylopia, garlic and black pepper tested positive for the presence of phenols, alkaloid, glycoside, terpenoids, steroid, flavonoids, Tannins and reducing sugar (Table 4.1). Glycoside was not detected in the aqueous extract of garlic. Also, terpenoids was not detected in the aqueous extract of black pepper.

Table 4.1. Phytochemical constituents of xylopia, garlic and black pepper.

	Aqueous Extracts of:		
Phytochemical	Xylopia	Garlic	Black pepper
Phenols/Polyphenols	+	+	+
Alkaloid	+	+	+
Glycoside	+	-	+
Terpenoids	+	+	-
Steroid	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Reducing sugar	+	+	+

(+) present/detected, (-) not detected.

4.2. Initial APC and pH of Fresh Pork

The initial microbial load and pH of *Longissmus dorsi, Tricep and Semimembranosus* before treatments were imposed are presented in Table 4.2. Significant differences (P< 0.05) in APC and pH were observed among the muscle types with a mean of 5.77 log CFU/g and 5.17 respectively.

Table 4.2. Initial microbial load on fresh loin, shoulder and topside muscles of pork.

Muscle Type	logCFU/g	pH
Semimembranosus	6.1 [a]	5.3 [a]
Tricep	5.8 [b]	5.2 [ab]
Longissmus dorsi	5.4 [c]	5.0 [b]
Lsd (0.05)	0.21	0.29
Mean	5.77	5.17

Values in the same column with different superscripts are significantly different (P<0.05).

4.3. Antimicrobial Activity of Aqueous Extracts of Spices

The inhibitory effects of xylopia, garlic and black pepper extracts at three concentrations including mixtures (equal combination of the three extracts), also with control and vinegar (standard bacteriostatic agent) treatments on the initial microbial load of pork are shown in Table 4.3. Statistical analysis indicated significant reductions (P< 0.05) in the initial microbial counts

of pork for the xylopia, garlic, black pepper, the mixture and vinegar treated pork in comparison with the control. No differences were observed in the initial microbial counts between the mixture (50g/500ml) and Vinegar treated samples. For all the pork samples, Aerobic Plate Counts (APC) (log10 CFU/g) increased during the storage period. The increase in storage time produced significant proliferations in APC, regardless of the treatment conditions (Fig 4.1). The initial (day 0) mean of APC (log10 CFU/g) for the control pork samples was 5.77. After one day of storage, the APC of the control meat reached a log mean count of 7.03, which is above the maximum recommended limit (7 $\log_{10}$ CFU/g) set by ICMSF (1986) for APC in processed meat. On the third day of storage, APC for untreated pork increased to 7.47 $\log_{10}$ CFU/g, while signs of spoilage started to appear as a slight foul smell. On the other hand, samples treated with spices and vinegar showed delayed microbial growth in comparison with controls. On the fifth day of storage, APC of garlic (50g/500ml) and black pepper (50g/500ml) treated pork samples recorded a log mean count of 6.90 and 6.83 respectively, which are close to the maximum recommended limit, during which signs of spoilage start to appear. On the seventh day of storage, the xylopia, mixture and vinegar treated samples recorded mean APC of 6.96, 6.6 and 6.87 respectively versus 9.17 for the control (Fig 4.1) while by the end of storage period (day 9), the xylopia, mixture and vinegar treated samples had mean APC values of 7.5, 6.9 and 7.3 respectively versus 9.80 in control (Fig 4.1). At day 9 of storage, samples containing mixture of spices (50g/500ml) had a lower APC (6.53 $\log_{10}$CFU/g) than the maximum recommended limit, while the control samples exhibited a higher count of 9.80 $\log_{10}$CFU/g that is above the maximum recommended limit. The data also showed that for day 0, 1, 3 and 5 of storage, the APC of standard (vinegar) was lower than that of the mixture (equal combinations of extracts of spices). However, for day 7 and 9, the APC of meat samples treated with the mixture of spices was lower

than that of the vinegar (Fig 4.1). The data shows that the aqueous extracts of xylopia are more potent than that of garlic and black pepper in reducing microbial load on meat surface during storage. Plate 4-1 shows the pictures of microbial load on meat samples treated with xylopia, black pepper, garlic, mixture, and vinegar as well as the untreated meat (control).

4.4. Effect of Extract Concentration on Microbial Load on the Surface of Pork

The concentration of extracts of spices significantly affected the microbial growth on pork meat surface (Table 4.3).In general, the APC (log_{10}CFU/g) decreased as the concentration of the extracts increased from 20g/500ml to 50g/500ml (Table 4.3). The 50g/500ml concentration for xylopia, garlic, black pepper as well as that for the mixture at 50g/500ml significantly ($P<0.05$) reduced the microbial load on samples of pork during the entire 9-day storage period compared with those for the 20g and 30g/500ml spice extracts (Table 4.3).

Table 4.3. Microbial load on pork samples treated with spices after the days of storage

	log_{10}CFU/g change after storage Day(s)				
Treatment	1	3	5	7	9
Control	7.03 [a]	7.47 [a]	8.30 [a]	9.17 [a]	9.80[a]
Vinegar	5.20 i	5.50 [m]	5.97 [l]	6.87[defg]	7.33[j]
Xylopia (20g/500ml)	6.20 [de]	6.43 [e]	7.00 [f]	7.33 [bcdef]	7.83[e]
Xylopia (30g/500ml)	5.90 [f]	6.10 [h]	6.57 [j]	6.93 [cdefg]	7.47 [i]
Xylopia (50g/500ml)	5.63 [g]	5.87 [j]	6.27 [k]	6.63 [efg]	7.20 [k]
Garlic (20g/500ml)	6.53 [bc]	6.87 [c]	7.40 [c]	7.70 [bc]	8.17 [c]
Garlic (30g/500ml)	6.37 [cd]	6.63 [d]	7.20 [d]	7.37 [bcde]	7.77 [f]
Garlic (50g/500ml)	6.00 [ef]	6.30 [g]	6.90 [g]	7.10 [bcdef]	7.50 [h]
Black pepper (20g/500ml)	6.67 [b]	6.93 [b]	7.50 [b]	7.77 [b]	8.23 [b]
Black pepper (30g/500ml)	6.50 [bc]	6.63 [b]	7.07 [e]	7.47 [bcd]	7.93 [d]
Black pepper (50g/500ml)	6.17 [de]	6.37 [f]	6.83 [h]	7.23 [bcdef]	7.67 g
Mixture (20g/500ml)	5.60 [g]	5.93 [i]	6.67 [i]	6.97 [cdefg]	7.33 [j]
Mixture (30g/500ml)	5.47 [gh]	5.70 [k]	6.27 [k]	6.57 [fg]	6.93 [l]
Mixture (50g/500ml)	5.27 [hi]	5.40 [m]	5.87 [m]	6.27 [c]	6.53 [m]
Lsd (0.05)	0.21	0.019	0.017	0.77	0.026
S.e.	0.04	0.01	0.14	0.46	0.03

Values in the same column with the same superscripts are not significantly different (P<0.05).

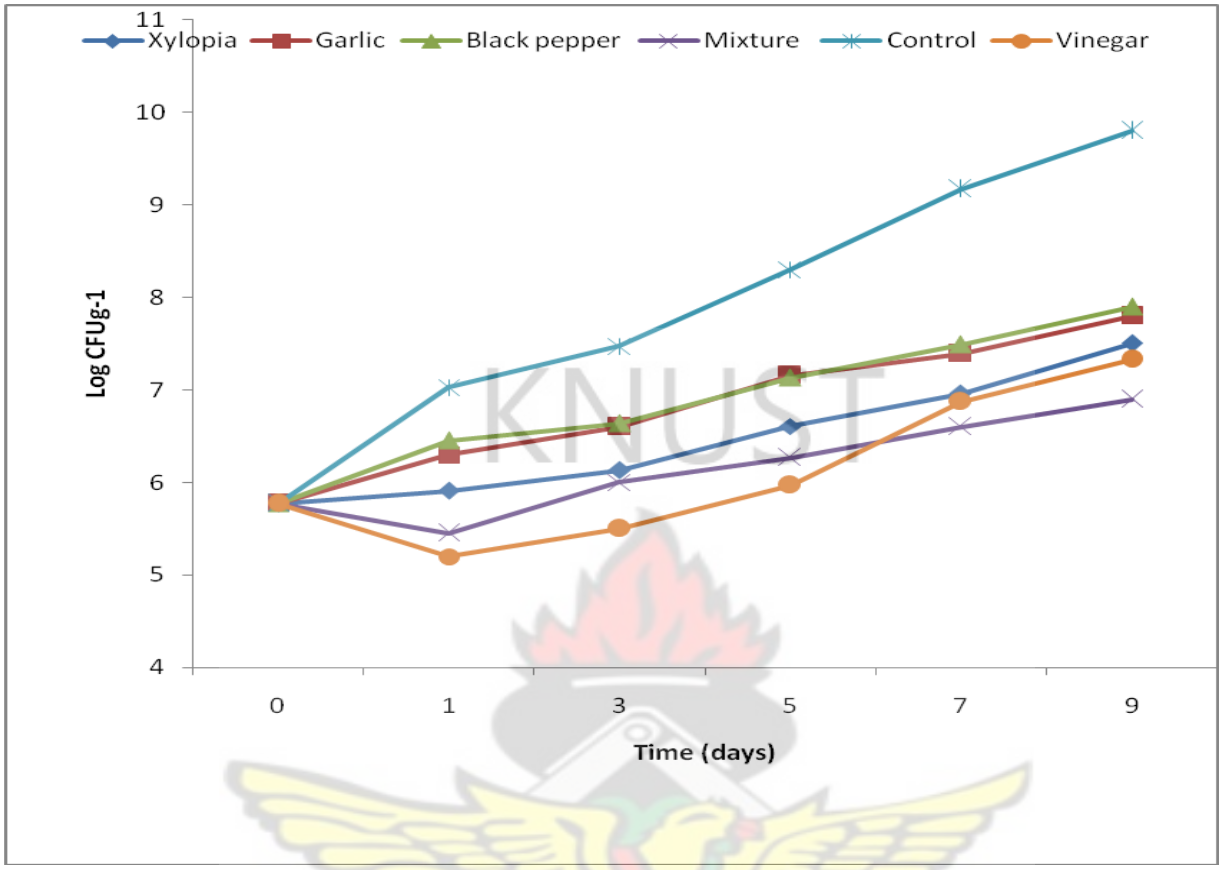
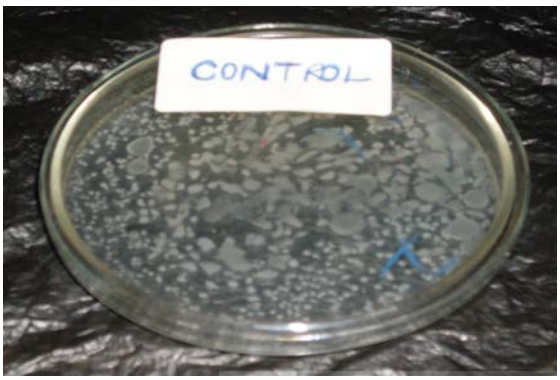
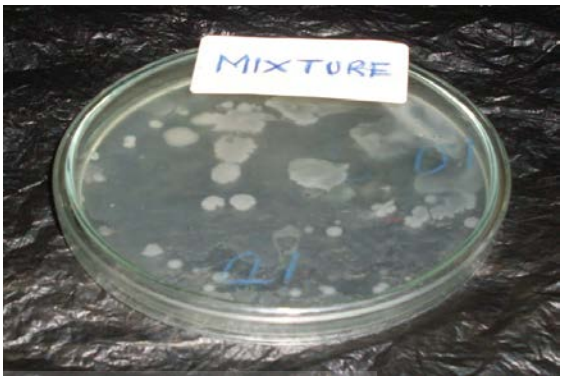


Figure 4.1. APC (log10 CFU/g) changes of fresh pork treated with three spice,mixture of spices and vinegar during 9 days of storage at room temperature.



a



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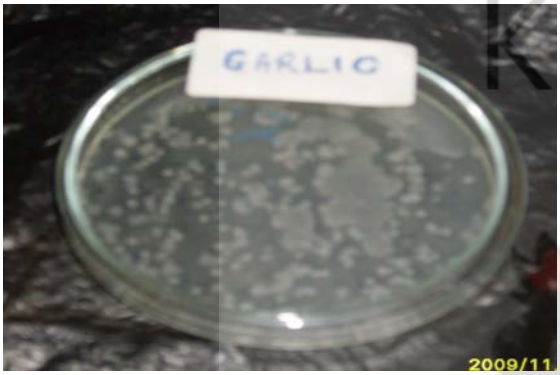

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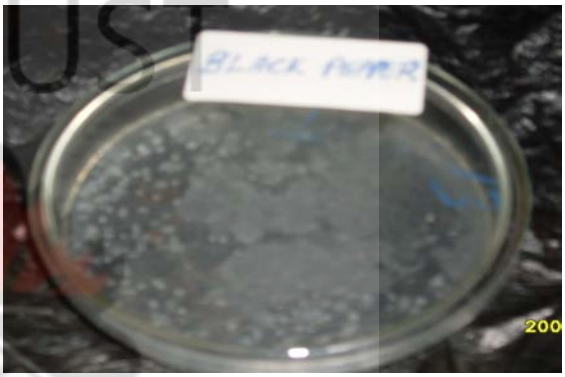

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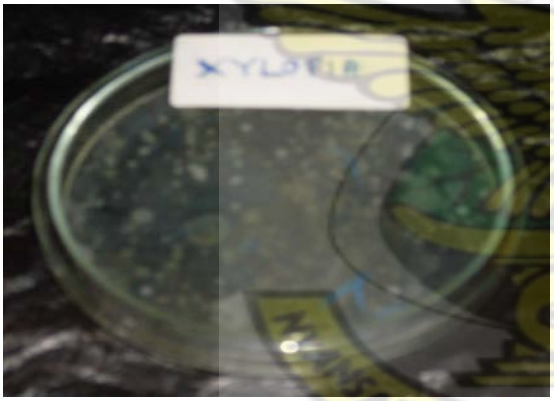

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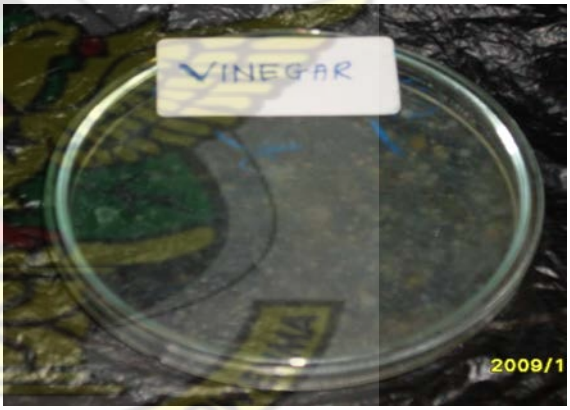

f

Plate 4.1. Colony forming units on plates with control (a) and samples treated with mixture (b), garlic (c), black pepper (d), xylopia (e) and vinegar (f). Take note of the numerous Colony Forming Units (CFU) on the control plate and fewer CFU on the mixture plate.

4.5. pH Measurements of Various Treated Pork Samples

The initial mean pH value of fresh pork samples was 5.17 (Table 4.1). Table 4.4 presents changes in pH values in fresh pork during storage at room temperature (23 ºC). The table results indicated that on the first day of storage, the pH values of all samples were similar, ranging from 5.44 to 6.57. After 9 days of storage however, no significant differences ($P<0.05$) in the pH values of the meat samples treated with spice extracts including the control and the standard (vinegar) were observed. On the third and ninth days, there were no significant difference among samples treated with the spices and the samples treated with vinegar. Throughout the storage period, the pH values of fresh pork treated with the three natural extracts increased slightly, whereas those of the control increased significantly (Fig 4.4).

Significant differences between the control and all treatments were observed after three, five, seven and nine days of storage (the pH values of all treatments ranged from 5.53 - 6.29, while that of the control ranged from (6.19 - 6.80).

Table 4.4. pH changes of pork treated with three spices and vinegar at room temperature.

	pH change on day(s) of storage				
Treatment	1	3	5	7	9
Control	6.04	6.19 [a]	6.07 [a]	6.60 [a]	6.80 [a]
Vinegar	5.44	5.59 [b]	5.63 [e]	5.86 [de]	6.15 [bc]
Xylopia (20g/500ml)	5.59	5.63 [b]	5.73 [c]	5.83 [ef]	5.93 [bc]
Xylopia (30g/500ml)	5.53	5.59 [b]	5.73 [c]	5.79 [fg]	5.86 [c]
Xylopia (50g/500ml)	5.45	5.58 [b]	5.70 [cd]	5.74 [h]	5.85 [c]
Garlic (20g/500ml)	6.57	5.73 [b]	5.73 [c]	5.83 [ef]	6.16 [bc]
Garlic (30g/500ml)	5.64	5.66 [b]	5.73 [c]	5.80 [fg]	6.12 [bc]
Garlic (50g/500ml)	5.50	5.66 [b]	5.69 [cd]	5.76 [gh]	6.05 [bc]
Black pepper (20g/500ml)	5.71	5.73 [b]	6.12 [a]	6.09 [b]	6.14 [bc]
Black pepper (30g/500ml)	5.70	5.73 [b]	5.85 [b]	6.03 [c]	6.29 [b]
Black pepper (50g/500ml)	5.64	5.70 [b]	5.80 [b]	5.89 [d]	6.21 [bc]
Mixture (20g/500ml)	5.56	5.60 [b]	5.70 [cd]	5.79 [fg]	5.89 [bc]
Mixture (30g/500ml)	5.56	5.60 [b]	5.69 [cd]	5.76 [gh]	5.84 [c]
Mixture (50g/500ml)	5.49	5.53 [b]	5.65 [de]	5.76 [gh]	5.79 [c]
Lsd (0.05)	n.s.	0.40	0.017	0.49	0.026
S.e.	0.15	0.17	0.14	0.16	0.03

Values in the same column with the same superscripts are not significantly different (P<0.05).

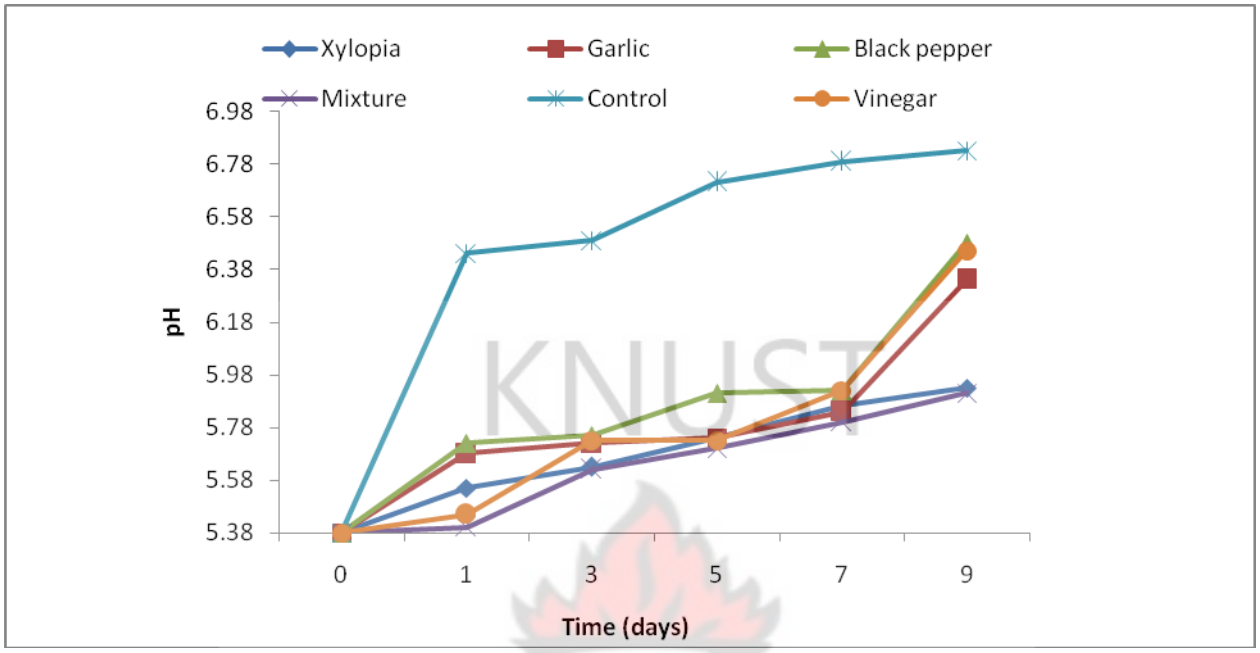

Figure 4.2. pH changes of fresh pork treated with three spices and vinegar during 9 days of storage at room temperature.

4.6. Influence of Pork Muscle Type on APC and pH Changes

The effect of muscle types and time of storage on the APC changes is presented in Fig 4.3. Significant differences ($P< 0.05$) in the interaction of *Longissmus dorsi, Tricep and Semimembranosus* on the number of colony forming units throughout the period of storage were observed. Log_{10} CFU/g of *Longissmus dorsi* was significantly lower than log_{10}CFU/g of *trice*p and *Semimembranosus*. In all the muscle types, microbial counts increased during the storage period. There was no significant difference in log_{10}CFU/g of *Tricep* and *Semimembranosus* on the ninth day of storage.

Fig 4.4 illustrated the effect of muscle types and time of storage on the pH changes. There were significant differences (P< 0.05) in pH of *longissmus dorsi, tricep* and s*emimembranosus* throughout the period of storage . pH of *longissmus dorsi* was significantly lower than pH of *tricep* and *semimembranosus* on the first, third, fifth and seventh days of storage. pH changes in *longissmus dorsi* was however similar to that of *tricep* on day 0 and day 9 of storage.

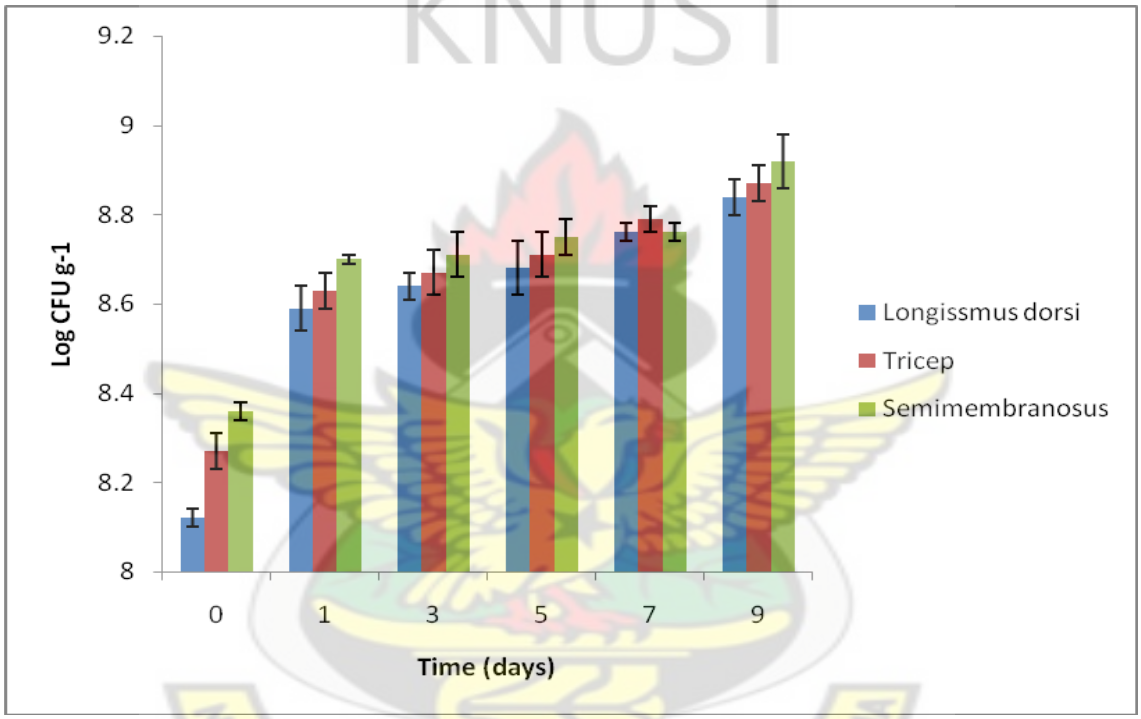

Fig 4.3. Changes in APC of *Longissmus dorsi, Tricep* and *Semimembranosus* muscles during 9 days of storage at room temperature.

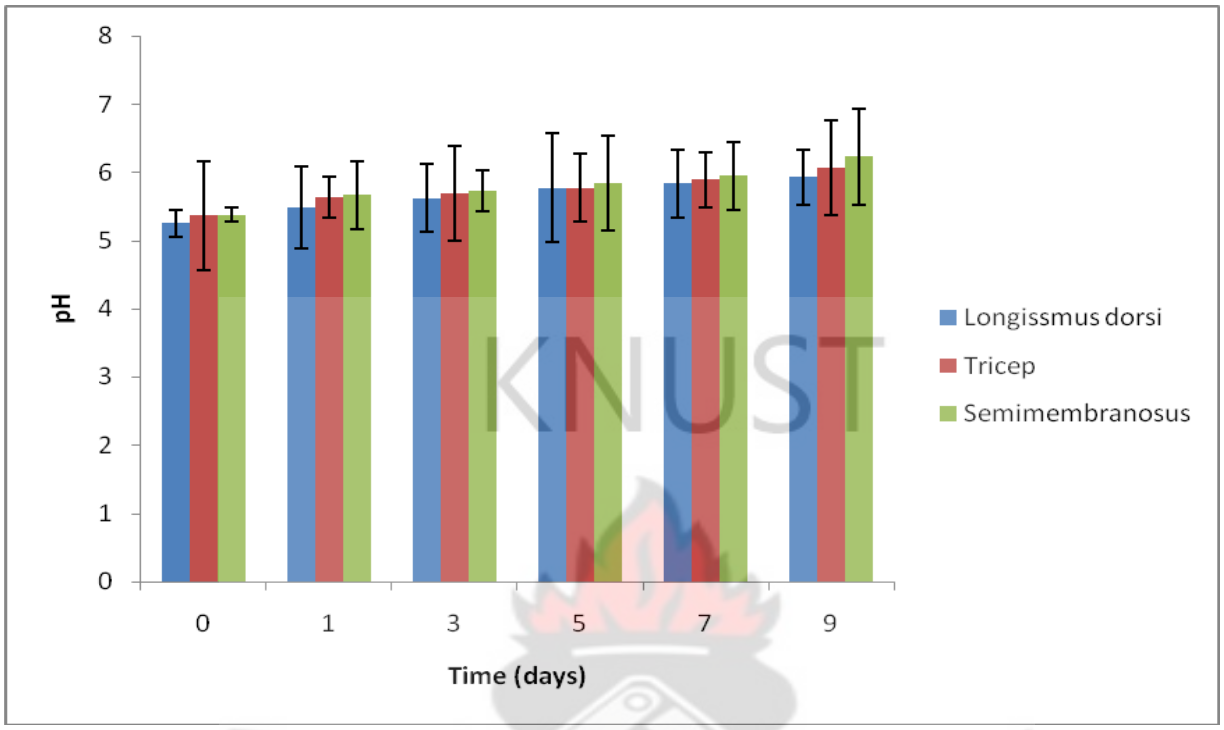

Fig 4.4. Changes in pH of *Longissmus dorsi, Tricep* and *Semimembranosus* muscles during 9 days of storage at room temperature.

4.7. Colour Changes

The spice extracts had an immediate effect on the colour parameters of the samples after treatment in comparison with the control (Table 4.5). During 9 days of storage, the redness of the treated samples were basically steady (slightly changed) in comparison with a considerable decrease in redness of the untreated meat sample.

4.8. Sensory Properties of Preserved Pork

Sensory properties of samples were evaluated by a taste panel consisting of 10 students with a mean age of 23 years and the results are summarized in Table 4.6. Overall, the sensory scores of

Mixture spice-treated sample (50g/500ml) was the best, followed by the Vinegar treated meat, Xylopia treated meat (50g/500ml), Garlic treated meat (50g/500ml) and Black pepper treated meat (50g/500ml). The control samples were also equally accepted but ranked last. The major problem with all the treated samples was a loss of meaty flavour.

Table 4.5. Changes in colour and odour.

	Day(s) of Storage											
	0		1		3		5		7		9	
Treatment	S. C	odour	S.C	odour	S.C	odour	S.C	odour	S.C	odour	S.C	odour
Control	PR	-	BR	-	DB	+	DB	+	D	+	D	+
Vinegar	PR	-	PR	-	BR	-	BR	-	DB	+	D	+
Xylopia(20g/500ml)	PR	-	PR	-	BR	-	DB	+	DB	+	D	+
Xylopia(30g/500ml)	PR	-	PR	-	BR	-	DB	+	DB	+	D	+
Xylopia(50g/500ml)	PR	-	PR	-	PR	-	BR	-	DB	+	D	+
Garlic (20g/500ml)	PR	-	PR	-	BR	-	DB	+	D	+	D	+
Garlic (30g/500ml)	PR	-	PR	-	BR	-	DB	+	D	+	D	+
Garlic (50g/500ml)	PR	-	PR	-	PR	-	BR	-	DB	+	D	+
B. pepper (20g/500ml)	PR	-	PR	-	BR	-	DB	+	D	+	D	+
B. pepper(30g/500ml)	PR	-	PR	-	BR	-	DB	+	D	+	D	+
B. pepper(50g/500ml)	PR	-	PR	-	DB	-	DB	+	DB	+	D	+
Mixture(20g/500ml)	PR	-	PR	-	PR	-	BR	-	DB	+	D	+
Mixture(30g/500ml)	PR	-	PR	-	PR	-	BR	-	BR	-	DB	+
Mixture(50g/500ml)	PR	-	PR	-	PR	-	PR	-	BR	-	BR	-

Minus sign (-) = no change in odour; Plus sign (+) = positive formation of bad odour

PR- Purplish red, BR- brown red, DB- Dark brown, D- Dark, S.C-Sample colour

Table 4.6. Overall liking of cooked samples of preserved pork

Treatment	Overall liking	Acceptance rank
Control	1.4 [a]	14
Vinegar	6.3 [b]	2
Xylopia (20g/500ml)	3.1 [c]	9
Xylopia (30g/500ml)	3.2 [c]	8
Xylopia (50g/500ml)	5.7 [d]	4
Garlic (20g/500ml)	2.7 [e]	12
Garlic (30g/500ml)	3.0 [f]	10
Garlic (50g/500ml)	4.8 [g]	5
Black pepper (20g/500ml)	2.0 [h]	13
Black pepper (30g/500ml)	2.8 [e]	11
Black pepper (50g/500ml)	4.2 [i]	6
Mixture (20g/500ml)	4.0 [i]	7
Mixture (30g/500ml)	5.8 [d]	3
Mixture (50g/500ml)	7.2 [j]	1
Lsd	2.93	
S.e.d.	3.24	

Mean values with different superscripts are statistically different ($P<0.05$).

CHAPTER FIVE

5.0. DISCUSSION

Chemical analysis of the three natural extracts indicated that the major compounds present were polyphenols/phenols, alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar. Gas chromatographic –mass spectrometric analysis of the fruit of *Xylopia aethiopica* has been done by Noujou *et al.*, (2007) and revealed more than sixty compounds with 47.5-84.0% of monoterpenes hydrocarbon, mainly B-pinene and B-phellandrene + 1,8-cineole, 6.5-12.9% of oxygenated monoterpenes, 13.8-30.4% of sesquiterpenes, 0.4-0.6% of a minor diterpene identified as ent-13-epi manoyl oxide. Other studies indicated presence of phenolic oxides, flavonides, peperine, S-3-Carene, B-caryophyllene (Sherman *et al* (1997) in black pepper and allicin and sulfur-containing compounds such as diallyl sulfide and diallyl disulfide (Ankri and Mirelman, 1999) and Rabinkov *et al.*, (1998) in garlic.

Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials. In the commercial preservation of fish and fish products, natural antimicrobials from plant sources have been found to extend shelf life and preserve fishy taste and flavour (Martos *et al.*, 2007). In the present study, xylopia, garlic and black pepper aqueous extracts were found to reduce microbial load and extend the shelf life of pork.

The high initial microbial load on fresh pork from the abattoir is an indication of poor hygienic conditions in the Kumasi abattoir. Zakpaa *et al.*, (2009) also reported a similar observation on meat products of some selected markets in the Kumasi metropolis in Ghana.

The initial microbial counts of xylopia, garlic and black pepper treated pork in comparison with the control shows that the extracts of the three spices had antimicrobial activities on microbes on the surface of pork. This agrees with the results of Marie-Josee *et al.*, (2001) who reported

antimicrobial effect of extract of Mustard on microorganisms in chicken meat stored for two weeks at a none restrictive growth temperature of 22 ºC. Ali *et al.,* (2007) also observed that chopped garlic in ground beef and raw meat ball showed antimicrobial effects. Bahk *et al.,* (1990) reported that microbial inhibition by plant extracts could be manifested by an extended lag phase, increased generation time and decreased maximum growth or various combinations of these effects. In the present study, the maximum growth of the microbes growing on the pork was decreased and their lag phase prolonged by the xylopia, garlic and black pepper extracts. Billing and Sherman, (1998) reported that spices could kill microorganisms or inhibit their growth before they could produce toxins and suggested that the use of spices might reduce foodborne illnesses and food poisoning.

The 7.03 $\log_{10}$CFU/g recorded for control which is above the maximal recommended limit (7 $\log_{10}$ CFU/g) set by ICMSF (1986) for APC in processed meat and the occurrence of signs of spoilage on the third day of storage suggest a shelf life of 1-2 days for the control (pork).The occurrence of signs of spoilage and APC of 6.90 and 6.80 on garlic and black pepper respectively on the fifth day of storage indicates that garlic and black pepper extracts extended the shelf life of pork stored in room temperature to 5 days in comparison with the control which gives a shelf life of only 1 day of storage. Addition of garlic has been reported to produce significant reduction in growth of APC in chicken (Sallam and Samejima, 2004). Babu *et al*., (2000) also reported black pepper has extended shelf life of chicken to 6 days as compared to the control.

The mean APC of 6.96 and 6.87 for xylopia and vinegar (acetic acid) treated samples on the seventh day of storage suggests that xylopia and vinegar extended the shelf life of pork to 7-8 days in comparison with the control which had a shelf life of only one day. Shimoji *et al* (2004)

reported that addition of vinegar, a bacteriostatic chemical reduced microbial load and extended shelf life of beef. Singh and Malti (2009) reported that the use of bacteriostatic chemicals to preserve meat has side effects of chemical residue toxicity leading to cardiovascular diseases.

The extension of shelf life of pork treated with xylopia to seven days, similar to the extension limit of vinegar suggests that xylopia; a natural plant product could be used instead of vinegar, to extend shelf life of pork. The use of such natural additives has the extra advantage of the health benefits associated with the extracts. The antioxidant activity of grape seed extracts has been linked to boosting cardiovascular health by limiting oxidation of LDL (bad) cholesterol, while pycnogenol has been claimed to have beneficial effects on a wide range of medical conditions from diabetes to asthma, from boosting male fertility to improving the memory of mice (Sasse *et al,* 2003).

At the end of storage period (9 days), samples treated with combination of xylopia, garlic and black pepper had a lower APC (6.9 $\log_{10}$CFU/g) than the maximum recommended limit. This indicated that storage at room, xylopia and vinegar treated samples had shelf life of 7 and 8 days and the mixture treated samples had 9 days. This demonstrates that such a combination is more effective in reducing microbial load and extending shelf life of meat than using one of the spices alone. This result might have been due to the synergistic effect of the three spices studied. This finding is consistent with reports of Ziauddin *et al.,* (1996) who observed that many spices exhibit greater antibacterial potency when they are mixed than when used alone. Some are combined so frequently that the blends have acquired special names. An intriguing example is the French "quatre epices" (pepper, cloves, ginger, and nutmeg), which is often used to make sausages. Pepper and lemon (and lime) juice are among the most frequently used spices which suggest that pepper and citric acid play special roles-that is, as synergists. Citric acid potentiates

the antibacterial effects of other spices because low pH disrupts bacterial cell membranes (Booth and Kroll, 1989).

The initial higher APC of mixture than for the vinegar and the subsequent lower APC of mixture than vinegar during day 7 and 9 of storage shows that mixture was more stable in breaking down from the surface of pork and therefore stays for a long period while vinegar breaks down quickly. Hence the extracts of mixture of the three spices were found to be comparatively more efficacious than synthetic chemicals. This finding is in accordance with that of Sasse *et al.,* (2003) who reported that grape seed extract exhibited lower APC than NaL up to day 8 of storage at 23 ºC.

In the present study, the extracts of the three spices were characterized by presence of polyphenols. These phenolic compounds might predominantly contribute to the antimicrobial activities of the tested spices. The partial hydrophobic nature of phenolic compounds may degrade the cell wall, interact with the composition of and disrupt the cytoplasmic membrane-integrated enzymes, which may eventually lead to cell death (Shan *et al.,* 2007). In a similar study (Recep *et al*., 2008), the extract of *Eucalyptus radiata* with a high content of oxygenated monoterpenes (70.2%), was assayed against 33 pathogenic fungi and 30 bacterial strains. It exhibited considerable antibacterial and antifungal activities against most pathogens. These activities were attributed to oxygenated monoterpenes such as camphor, 1,8-cineole and terpinen-4-ol, which were reported to have antibacterial activities (Recep *et al.,* 2008, Pattnaik *et al*., 1997, Saban *et al*., 2005). Recent studies (Trombetta *et al*., 2005, Cristani *et al*., 2007) have speculated that the antimicrobial effect of terpenes may be partially due to perturbation of the lipid fraction of bacterial plasma membranes, resulting in alterations in membrane permeability and leakage of intracellular materials. Besides being related to the physiochemical characteristics

of drugs (such as lipophilicity and water solubility), as cited by the previous authors (Cristani *et al*., 2007 and Trombetta *et al*., 2005), this effect appears to be dependent on the lipid composition and net surface charge of the bacterial membranes. Disruption of the membrane by terpenes has also been shown in gram-positive (Cox *et al*., 2000; Ultee *et al.*, 1999) and gram-negative (Helander *et al*., 1998) bacteria.

The microbes on the surface of pork were most inhibited when the concentration of the extracts was 50g/500ml. This showed that 50g/500ml concentration of the extracts contain more active ingredients than 20g/500ml and 30g/500ml concentrations. This agrees with some workers who observed that factors like the concentration of extracts could influence the active ingredients present in the extract (Devant *et al*, 2000).

The slight increase in the pH of fresh pork treated with the three natural extracts and the significant increase in the pH of the control show that the spice extracts contained high levels of phenolic compounds, which contributed to lower pH values in fresh pork and were probably involved in the maintenance of meat colour through their antioxidant effects. As the final pH increased, the meat gradually became darker. High final pH also affected the colour stability of the fresh meat, probably because it affected enzyme activity and the rate of oxygenation. Reducing enzymes are necessary to convert metmyoglobin back to oxymyoglobin, which is well known to contribute to the colour of fresh meat (Pietrasik *et al*., 2006). Generally speaking, the shelf life of meat and meat products will be longer when the pH is lowered (Cross and Overby, 1998).

Studies on meat colour often focus on the redness, because the redness of meat is an important component of visual appeal to consumers (McCarthy *et al*., 2001; and Rhee *et al*., 2003). Several authors have studied the colour of meat products and reported that meat oxidation caused a

decrease in redness (Higgins *et al*., 1998; Lee and Ahn, 1998). To some extent, the present study revealed the protective effects of spice extracts against decrease in redness of raw pork during storage. Palanisamy *et al.,* (2008) reported an antioxidant activity of extracts from the bark of *Chamaecyparis obtuse* var. *formosana.* Although the addition of spice extracts caused changes in the meaty flavour of pork, these changes may be acceptable to consumers, because many spice extracts are traditionally used for curing meat in Ghana.

The significant differences in the loin, shoulder and topside muscles of pork in microbial growth and pH shows that the muscle types differ in their susceptibility to microorganisms growing on the surface of pork. Cross and Overby ,(1998) stated nutrient content, antimicrobial constituents, pH value or degree of acidity of meat, water activity(a_w) value as endogenous factors influencing the microbial growth in meat and meat products. Boles and Pegg, (2000) also observed that different myoglobin concentrations are found in the various muscles of cattle. These endogenous factors might be responsible for the differences in microbial growth and pH of loin, shoulder and topside muscles observed in this study. This finding suggests that preservation of different muscles together should be discouraged, since some muscles might spoil earlier and contaminate others and thus shortening shelf life of meat.

The overall acceptability of mixture (50g/500ml) as the best by the taste panel might be due to the synergistic effect of xylopia, garlic and black pepper extracts in reducing microbial load. Also, the slight change in redness of mixture (50g/500ml) could be due to high a high antioxidant property of the combination of xylopia, garlic and black pepper.

CHAPTER SIX

6.0. SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The findings of the study are summarized as follows:

1. Phenols, alkaloids, glycoside, terpenoids, steroid, flavonoids, tannins and reducing sugar were the major phytochemicals in xylopia, garlic and black pepper.

2. The extracts of xylopia, garlic and black pepper had antimicrobial activities on microbes on the surface of pork.

3. The pork without any treatment (control) had a shelf life of 1-2 days.

4. Garlic and black pepper treated pork samples had a shelf life of 5 days.

5. Xylopia and vinegar (acetic acid) dipped pork samples had a shelf life of 7-8 days.

6. The mixture (equal parts of xylopia, black pepper and garlic) extended the shelf life of pork samples to 9 days suggesting the synergistic effect of the three spices.

7. The microbes on the surface of pork were most inhibited when the concentration of the spice extracts was 50g/500ml.

8. There was a slight increase in the pH of pork treated with the three extracts and significant increase in the pH of the control after day one.

9. The meat samples became darker as the pH increased.

10. There was a significant difference in pH and microbial load values of *Longissmus dorsi, Tricep* and *Semimembranosus* muscles of pork.

11. The mixture of spice (50g/500ml) treated pork samples was the best of all the treated meat accepted by the taste panel.

6.2. Conclusion

The natural extracts from xylopia, garlic and black pepper applied to fresh pork could help reduce the microbial load and decrease pH during storage at ambient temperature for up to 9 days. Such natural extracts contain high levels of bioactive phenolic compounds that can help to control foodborne pathogens and inhibit lipid oxidation. Xylopia alone and mixture of xylopia, garlic and black pepper exhibited the most effective antibacterial property and lowest pH activity. In comparison with synthetic additives, natural extracts may be more acceptable to consumers and regulatory agencies and also potentially benefit human health. Therefore such natural extracts might be used as multifunctional preservatives in meat.

6.3. Recommendations

On the basis of the findings of this study, it is recommended that:

1. The experiment should be repeated using specific strains or isolates of microbes on the pork meat surface.

2. Further studies addressing extraction of the compounds in the spices and testing them against microbes on the surface of pork meat will be necessary.

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8. 0. APPENDIX

Appendix 1: Analysis of variance table for colony forming units on day 1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.0003762	0.0001881	0.95	
rep.*Units* stratum					
Treatment	13	0.2112286	0.0162484	81.92	<.001
Residual	26	0.0051571	0.0001984		
Total	41	0.2167619			

Appendix 2: Analysis of variance table for colony forming units on day 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.0001000	0.0000500	0.30	
rep.*Units* stratum					
Treatment	13	0.2978000	0.0229077	138.51	<.001
Residual	26	0.0043000	0.0001654		
Total	41	0.3022000			

Appendix 3: Analysis of variance table for colony forming units on day 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.0002476	0.0001238	0.33	
rep.*Units* stratum					
Treatment	13	0.2281333	0.0175487	46.79	<.001
Residual	26	0.0097524	0.0003751		
Total	41	0.2381333			

Appendix 4: Analysis of variance table for colony forming units on day 7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.002414	0.001207	1.00	
rep.*Units* stratum					
Treatment	13	0.187667	0.014436	11.98	<.001
Residual	26	0.031319	0.001205		
Total	41	0.221400			

Appendix 5: Analysis of variance table for colony forming units on day 9

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.0002048	0.0001024	0.78	
rep.*Units* stratum					
Treatment	13	0.1133619	0.0087201	66.78	<.001
Residual	26	0.0033952	0.0001306		
Total	41	0.1169619			

Appendix 6. Questionnaire for sensory evaluation of pork samples treated with plant spices.

The purpose of this questionnaire is to enable the student carry out a research as a partial fulfillment of her MSc course (Meat Science) in the Kwame Nkrumah University of Science and Technology. The information you provide on this form will be kept strictly confidential.

a) Personal Data

1. Age: 18-40 [ ], 41-60 [ ], 61- 80 [ ], > 80 [ ]

2. Sex: Male [ ], Female [ ]

3. Marital status: Single [ ], Married [ ], Separated [ ], Divorced [ ]

4. Educational level: None [ ], JSS [ ], Middle school [ ], Tertiary [ ]

5. Occupation: Student [ ], Trader [ ], Carpenter [ ], Teacher [ ], others [ ]

6. Tribe: Akan [ ], Ewe [ ], Ga [ ], Frafra [ ], Mamprusi [ ], others [ ]

7. Religion: Christianity [ ], Islam [ ], Traditional [ ], others [ ]

8. How do you preserve meat in the house?

Refrigeration [ ], Heat [ ], Store at room temperature [ ], Drying [ ],

Salting [ ], Any other [ ]

17. How long do you preserve them?

1-5 days [ ], 6- 10 days [ ], 1- 3 months [ ], 4- 6 months [ ], 7 + months [ ]

18. Will you like a better method of preservation? Yes [ ], No [ ]

If yes, can you suggest one?.....

10. To what extent would you accept the meat sample you have tasted?

1 [ ] strongly like, 2 [ ], 3. [ ] 4. [ ] 5. [ ] 6. [ ]

7. [ ], 8. [ ], 9 [ ] strongly dislike.

Appendix 7. An autoclave used for sterilization during the research.

Appendix 8. An incubator used to incubate the plates.