UNIVERSITY FOR DEVELOPMENT STUDIES

ASSESSMENT OF MICROBIAL LOAD OF LOCALLY PRODUCED MEAT (BEEF, PORK AND GUINEA FOWL MEAT) IN BOLGATANGA MUNICIPALITY

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DECLARATION

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere. Related works by others which served as a source of knowledge has been duly referenced and acknowledged.

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I hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Meat microorganisms are one of the main sources of food borne illnesses, posing serious challenges in developing countries including Ghana. This study determined the microbial load of 150 fresh and smoked meat samples (50 beef, 50 pork and 50 guinea fowl meat) collected from meat retail shops in the Bolgatanga Municipality. Beef samples were obtained from Stanbic, Starlife, Central mosque, Jolly hut, and Mobile clinic shops; pork samples were obtained from Soe, Atulbabisi, Pobaga, Zobisi, and Dagbew shops; guinea fowl samples were obtained from Atibire, Next door, Comeci, Speed link and Ojam shops. The surface of fresh and smoked meat samples were swabbed using cotton swabbed and stored under 4°C for transportation to the Spanish Laboratory of the University for Development Studies, Nyankpala. The meat samples were analyzed immediately on arrival at the Laboratory under aseptic conditions for total aerobic bacteria. The surrounding environments of the retail shops were also observed. Total aerobic count for smoked and fresh beef ranged from 4.75 — 6.58 log cfu/g, that of pork ranged from 4.33 — 6.94 log cfu/g and that of guinea fowl ranged from 4.90 — 6.73 log cfu/g. Smoked pork from Zobisi had the highest microbial load of 6.94 log cfu/g, followed by fresh beef (6.56 log cfu/g) from Jolly hut and fresh beef (6.52 log cfu/g) from Central mosque. Bacterial species identified on the fresh and smoked beef, pork and guinea fowl meat samples were *Staphylococcus spp., Escherichia coli, Streptococcus spp., Pseudomonas spp., Proteus spp., Klebsiella spp.* and *Bacillus spp*. Generally, among the fresh and smoked meat samples from retail shops, fresh beef and smoked guinea fowl meat were the most contaminated. Pork (smoke and fresh) samples were the least contaminated *Staphylococcus spp.* and *Escherichia coli* were the most common identified bacteria. Physical observation revealed that meat sellers were

involved in unhygienic practices such as using of knives without sterilizing them, wearing of dirty aprons and/or clothes, busily conversing, coughing, and sneezing while selling meat. The identification of *Staphylococcus spp.*, *Escherichia coli* and the other organisms on the fresh and smoked meat samples is an indication of the presence of pathogenic food borne microorganisms.

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DEDICATION

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LIST OF ACRONYMS/SYMBOLS

Cfu/g	Coliform units per gram
CF SAN	Center for Food Safety and Applied Nutrition
DOA	Death on arrival
E^h	Redox potential
EU	European Union
EUROPA	European Union's Web portal accessible
FAO	Food and Agriculture Organization
FDA	Food and Drugs Administration
GIT	Gastro intestinal tract
GSS	Ghana Statistical Services
HACCP	Hazard Analysis and Critical Control Point
H202	Hydrogen peroxide
MAP	Modified Atmosphere Packaging
MSC	Meat Standard Committee
OIE	International Animal Health Organization
PH	Measure of acidity or alkalinity

PSE	Pale soft exudative
SADA	South Africa National Department of Agriculture
VP	Value Package
WHO	World Health Organization

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

Food-borne infections still remain one of the major problems of public health worldwide. Though data from different countries seem to report increases in the incidence of food-borne diseases, these data may not always represent the actual fact on the ground. Food production, processing and distribution in the world, differ from country to country. Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations which can cause its spoilage and food borne infections in human, resulting in economic and health losses (Komba *et al.,* 2012). Although muscles of healthy animals do not contain microorganisms, meat tissues get contaminated during the various stages of slaughter and handling *(Ercolini et al., 2006).*

A great diversity of microbes inhabits fresh meat, but different types may become dominant depending on pH, composition, textures, storage temperature, and means of transporting raw meat (Ercolini *et al.*, 2006; Li and Zhu 2006; Adu-Gyamfi *et al.*, 2012). In addition to protein, meat is a rich source of fat, low in carbohydrate with sufficient water activity that supports the growth of both spoilage and pathogenic bacteria. Major spoilage organisms in raw meat and poultry are Pseudomonas spp., Shewanella spp., Brochothrix spp. and members of Enterobacteriaceae.

Growth of yeasts and molds is essentially slow on fresh meat as compared to bacteria, therefore, they are not major component of spoilage flora (Doyle, 2007). The food and Agricultural organization (FAO) of the United Nations and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem and an important cause of reduced economic productivity (Kaferstein, 2003). Raw meat may harbour many important pathogenic microbes such

as Salmonella spp., Campylobacter jejuni/coli, Yesinia enterocolitica, E. coli, S. aureus and, to some extent, *Listeria monocytogenes,* making the meat a risk for human health, as without the proper handling and control of these pathogens, food borne illnesses may occur (Norrung *et al.,* 2009).

The meat available at retail outlets comes through a long chain of slaughtering and handling, where each step may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environment are major factors contributing to bacterial contamination of meat (Gill *et al.,* 2000). Contaminations can be compounded during transportation, storage and handling of meat at the butcher shops. Meat has high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth (Rao *et al.,* 2009). Apart from the possibility of microbial spoilage, meat at the point of sale may carry disease causing bacteria whose mere presence is of concern because the meat then becomes a vehicle for food poisoning outbreaks.

In Ghana, a number of abattoirs and meat processing units are operating without standard quality control systems. Meat is transported to the markets either with improvised meat vans, taxis, motor cycles or bicycles (Teye *et al,* 2006). Furthermore, meats are sold in the open sometimes with or without sieves, and on tables that are not well maintained or cleaned after work. This exposes the meat to a number of pathogens some of which may be pathogenic or non-pathogenic (Adzitey and Nurul 2011).

To control food-borne illnesses and to keep the microbial load of raw and processed meat in check, food safety requirements should be followed strictly in accordance with HACCP (Hazard analysis critical control point). In developing countries like Ghana, the abattoir environment, its sanitary level, transportation and storage conditions not only contaminated but also enhance the growth of different types of spoilage (Adzitey and

Nurul, 2011). The Present study was designed to assess the microbial load of local produced meat (beef, pork, guinea fowl meat) at retail outlets in different areas of the Bolgatanga Municipality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meat as food

Most meat for human consumption comes from domestic animals, including cattle, pigs, sheep, chickens, turkeys, ducks and rabbits. Meat is an important source of protein and a valuable commodity in resource poor communities. In many developing countries, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses of meat as well as by products from animal carcasses. Slaughtering places are frequently contaminated and may not be protected against dogs, rodents and insects. Meat products coming from such conditions often deteriorate due to bacterial contamination, especially in warm climates and in summer (Daft *et al*, 2003)

Meat is a nutritious food as the protein provides all essential amino acids in the proportionate amounts required by man and is also an excellent source of iron, thiamine, and niacin, phosphorus, potassium and sodium (Huda *et al.*, 2010; Rao et al., 2009). Schonfeldt and Welgemoed (1996) pointed out that meat is a valuable part of the human diet because (a) it is the most concentrated and good source of first class protein, thus, it contains those amino acids which are essential for human life; (b) it stimulates metabolism due to its high protein content, thus assists the body in the production of heat and energy; (c) it is satisfying, for the presence of fat in the diet delays emptying of the stomach (Marsland, 2003). According to Hoffman and Mellett (2005), in species like cattle, there is a move towards enhancing the quality of meat through the animal's diet. This will be relevant to those that will result in an animal

with healthier fat. Current research is based on the production of meat containing higher levels of conjugated linoleic acid (CLA).

2.2 Meat quality

It is a well-established fact that the older an animal the tougher the meat. Meat from old animals tends to be tougher than meat from young animals (Teye and Salifu, 2006). Wholesomeness is very important to the health of the consumer. Good meat may be recognized by a uniform colour, firm, elastic, texture moist to touch, scarcely perceptible and clean odour. Warriss (2000) defined meat quality on the basis of its conformational and functional qualities. The same author referred to the functional qualities as the desirable attributes in a product whilst the conformance qualities take into consideration producing products that exactly meets consumer's specifications. Post-slaughter animal handling has an adverse effect on meat quality. Adzitey and Nurul (2011) reported that poor carcass quality will definitely reflect in poorer meat quality. Post-slaughter meat handling begins at the abattoir (just after killing), and continues to process of meat (processing meat into various meat products), to the market (selling of meats) and finally to consumers (cooking and eating). Meat quality grade or level is determined by considering the degree of marbling and firmness in relation to the maturity of the carcass. There are eight grades or levels of meat, although only the first three or four are usually sold in markets United States Department of Agriculture (USDA, 1999). Kauffman and Eikelenboom (1990) also reported three levels of meat quality. The first level which has the highest priority requires the meat to be wholesome. It should be safe to eat and have nutritionally adequate levels of proteins, vitamins and minerals. The second level requires the meat to show minimum shrinkages during processing; including cooking and the third level requires the meat to have maximum attractiveness in terms of appearance, convenience

and eating quality. According to Teye and Salifu (2006) tenderness is an important factor in consumer's perception of meat quality.

2.3 Meat consumption

Several research findings indicate that a number of factors (animal welfare, environmental, safety, taste and health) influence the consumption of meats in any economy including Ghana, Metaly *et al.,* 2010; Damisa and Hassan 2009; Renuka *et al.* 2009; Liu and Deblitz 2007; McCarthy *et al.*, 2004; Gossard and York 2003; McCarthy *et al.,* 2003; Verbeke and Viaene, 1999; Zey and McIntosh, 1992. Various independent variables (income size and composition of the household, age, birthplace, education and employment status of the housekeeper, have also been found to influence the consumption of meat. Liu and Deblitz (2007) reported that economic and social as well as demographic variables such as price, urbanization, education, and presence of a child, safety, preference, changing lifestyles, and health concerns affect meat consumption at home in China.

According to Warriss (2010) humans are adapted to an omnivore's diet, based on the shape of their teeth and their unspecialized gut, and it is likely that quite early in human evolution meat began to play a part in our diet (Warriss, 2010). The important meat producing species remain domestic cattle, sheep, pigs, and poultry. Cattle, sheep and pig are often referred to as red meat species and poultry as white meat. The importance of the three red meat species in supplying meat protein differs in different parts of the world. Beef is most important in the North and South America, Africa and Europe, while sheep are most important in the Near East and pigs in the Far East (Warriss, 2010).

Red meat and poultry contribute about a sixth of all protein consumed by humans and if fish, milk and egg are included, animal products supply a third (Warriss, 2010). Not only is meat a very concentrated source of protein, but it also has a high biological value because its composition matches closely to that of our own proteins. It contains all the amino acids essential for human health. Meat is also an important source of the B vitamins, particularly B1 (thiamine), niacin (nicotinic acid), B2 (riboflavin), B6 and B12 (cyanocobalamin) and vitamin A (retinol) (Warriss, 2010). It is a major source of iron, copper, zinc, and some selenium (Warriss, 2010). The iron in meat has high bioavailability, the main reservoir being as a component of the haemprotein myoglobin. Iron deficiency is the common nutritional deficiency in the world (Warriss, 2010). In Ghana, meat consumption is mostly during Christmas and Islamic festivals and also during months prior to farming seasons (Teye and Salifu, 2006). The most important factor influencing meat purchase is finance. Also, age significantly affects meat consumption negatively. According to De Silva *et al.* (2010) when people become old they become more conscious of their health and nutrition and as such reduce the intake of some meat products, especially red meat. In a study by Reicks (2006) it was established that the three most important factors influencing the purchase of meat products are taste attributes, price, and product consistency. Ingram (2004) indicated that meat consumption is influenced by factors such as the wholesomeness of meat, quality of meat and the price of the meat. In a similar study Damisa and Hassan (2009) listed factors influencing the consumption of poultry meat as income, price, household size and education.

2.4 Abattoir

An abattoir is a "slaughter facility, whether stationary or mobile, at or on which animals are slaughtered or intended to be slaughtered (Meat Safety Act 40 of 2000). This will

include areas in or adjacent to such facilities, which will be where carcasses are chilled or meat or animal products are handled" (Meat Safety Act 40 of 2000). The slaughtering process is defined as "the killing of an animal and the performance of the usual accompanying acts in connection therewith in order to obtain meat and animal products there from" (Meat Safety Act 40 of 2000). According to Bekker (1998), an abattoir is the reverse of an assembling factory, where a pre-manufactured item, an animal is systematically dismantled to the primary components. The slaughtering process should be described from the point of holding live animals until chilling of the carcasses (Neetling, 2004).

23 The process of slaughtering and dressing of food animals

According to Bekker (1998), the process of slaughtering and dressing of food animals generally involves:

1. Receiving live animals to slaughterhouse and rested in the Holding Lairage and then sent to the Waiting Lairage on the day before slaughter.
2. Ante-mortem inspection - all animals are inspected by the Ante-mortem Inspection Unit of the Slaughterhouse (Veterinary) Section. Suspected diseased or injured animals are screened out for isolation slaughter.
3. Stunning - animals are rendered unconscious by either electric tong or gas or a shot from a pneumatic captive bolt into the brain.
4. Shackling and hoisting - the stunned animal is shackled by the left hind leg. It is then hoisted onto the overhead conveyor railing and pushed to the sticking point.
5. Sticking and Bleeding - a cut is made at the neck to sever a group of blood vessels including jugular veins. The animal is bled by passing slowly over the bleeding trough.

6. Removal of the head, legs and tail - the forelegs, the head and the tail are removed from the carcass (in cattle only). The tail and forelegs are put into a plastic bag to prevent contamination to the carcass. Whilst the head is hanged up for inspection.
7. Dehiding - in ruminants the hide is chained and pulled off at the flank by a hide puller.
8. Dehairing/ DE feathering- in pigs and poultry
9. Opening the brisket and evisceration - the brisket is cut by an electric saw. Offal is taken out and dropped onto the large moving viscera table.
10. Carcass splitting - the carcass is split longitudinally by an electric saw along the vertebral column into two halves.
11. Carcass and offal inspection - the carcass and offal are inspected by Health Inspectors. Only meat and offal that are fit for human consumption will pass the inspection and unfit meat / offal / parts will be condemned.
12. Stamping - the inspected carcass and offal which are fit for human consumption are officially stamped.
13. Rinsing of carcass and offal cleaning - the carcass is then rinsed in rinsing chamber, and offal is cleaned at offal washing room.
14. Quartering - each side of the beef carcass is cut into two quarters between the 5th and 6th ribs by mechanical scissors in the Quartering Area.
15. Meat delivery - quarters, offal and other parts of the animals are sent to the Meat Dispatch Bank, and then are collected and delivered by meat delivery vehicles to individual retail outlets.

2.6 Cruelty to animals

The welfare of animals is of interest to many people in most parts of the world. Concern about the way animals are treated depends on many factors, including socio-economic conditions, culture, religion and tradition (McCrindle, 1995; Wilkins *et al.*, 2005). Animals have to be killed to produce meat, or in connection with other farming activities, measures have to be taken to avoid unnecessary suffering, avoidable excitement, pain, or suffering during slaughter or killing and related operations, both inside and outside slaughterhouses (Gregory, 1998).

The International Animal Health Organization (OIE) has laid down welfare standards for the humane handling and slaughter of livestock. In 2008 the General Meeting also adopted a definition of animal welfare and reaffirmed the criteria for humane slaughter, long distance transport, as well as culling during disease outbreaks (OIE, 2004). The European Union (EU) stipulates in its animal welfare legislation that livestock must be killed in a way that avoids unnecessary suffering. Cultural and religious practices, as encountered in informal, unsupervised ritual slaughter, can present serious welfare problems as the animals are not correctly restrained and there are no pre-stunning procedures (Wilkins *et al.*, 2005).

The informal marketing of livestock in urbanized communities creates animal welfare problems due to ignorance, carelessness, lack of compassion and lack of proper facilities, especially in cases of illegal "bush" slaughtering. Kosher, halal and informal ritual slaughters in the African tradition are still issues of welfare concern (EUROPA, 2007). The five-freedoms form a basis on which an evaluation can be made of the welfare of the animal (good or bad) in any particular livestock production system:

- Freedom from thirst, hunger and malnutrition- thus ready access to fresh water and diet to maintain full health and vigor;

- Freedom from discomfort thus providing suitable environment including shelter and comfortable resting area;
- Freedom from pain, injury and disease by preventing or rapid diagnosis and treatment;
- Freedom to express normal behavior by providing sufficient space, proper facilities and company of the animals' own kind;
- Freedom from fear and distress by ensuring conditions to avoid mental suffering

(Wilkins *et al.*, 2005). In developing countries in Africa, animals for slaughter are transported on foot or on motorized transport that is not designed for animal transport. Animals that are transported by foot often walk for days without adequate rest, water or feed (Appleby *et al.*, 2008).

2.7 Risk associated with informal slaughter

Food provides an ideal medium for the growth and spread of a wide range of pathogens including cholera, botulism, shigellosis and typhoid fever. The informal food trade and the informal slaughtering of animals pose a public health threat due to inadequate hygiene. There is also a negative impact on the environment (Unc and Goss, 2004). Informal marketing also increases public health costs, in as much as products that do not comply with food safety norms pose high risks. The economic advantages to butchers of choosing the informal market include cost saving through lack of quality control and selling of meat and by-products that should have been discarded. In the particular case of the meat industry, the major financial advantage for the butcher, of choosing informal slaughter, is the use of animals that would otherwise have been rejected due to lack of quality. However, these cost savings that benefit the butcher may have direct consequences on public health (Abu-Samra *et al.*, 2007).

2.8 Impacts of informally slaughtered animals

2.8.1 Impacts on human health

Food-borne diseases constitute an important public health problem in both developed and developing countries, although the health and economic aspects are often obscured by an insufficiency of data (Tauxe, 1997; WHO, 1995). They are responsible for high levels of morbidity and mortality in the general population, particularly in high risk groups, such as infants, young children, the elderly and the immuno-compromised (WHO, 1995). While some developed countries have reasonably accurate data on the impact of food-borne diseases, it is rarely possible to derive similar statistics for developing countries because of a lack of surveillance systems for collecting reliable data (Schneider, 2004). It is therefore difficult to estimate what proportion of these diseases can be ascribed to eating contaminated meat, as most cases go to local clinics where treatment is given by nurses and few records are kept. The causes of deaths in rural areas of developing countries are seldom investigated, as autopsies are culturally unacceptable (McCrindle, 2004). In Ghana, there is very little information available on the true level of exposure of specific populations to potential hazards, particularly in the case of bacterial diseases transmitted by consumption of meat and meat products. Even at the international level, it is difficult to obtain accurate estimates of microbiological food-borne diseases. In England and Wales, food-borne diseases were responsible for 2,366,000 cases, with 21,138 hospitalizations, and 718 deaths (Adak *et al.*, 2002; Mead *et al.*, 1999).

2.9 Microbial quality of meat

The major bacterial agents causing food-borne diseases include *Campylobacter species, Escherichia coli, Listeria monocytogenes, Salmonella species* and *Staphylococcus aureus* (Singh and Prakash, 2008; Doyle and Erickson, 2006). Each of these bacterial

agents have uniquely adapted to the conditions established by current food production and distribution systems, and may easily be introduced into slaughter houses by farm animals that harbor them, food handlers or pests, thus contamination of meat may occur during processing (Gill and Hamer, 2004; Adams and Motarjemi, 1999). The slaughter process contributes to the prevalence of food borne pathogens through contamination of the carcass and cross-contamination between infected and uninfected carcasses (Horrocks *et al.*, 2009).

Spoilage by bacteria causes significant economic loss for food industries (Rodriguez-Calleja *et al.*, 2005). The microorganisms that have been identified as playing a major role in the onset of food spoilage belong to the genus *Pseudomonas*. These psychotropic *Pseudomonas species* pose a significant spoilage problem in refrigerated meat and meat products due to their ability to produce and secrete hydrolytic enzymes such as lipases and proteases (Nychas *et al.*, 2008; Ellis and Good acre, 2001). Healthy animals and poultry carry a very large and diverse micro flora that may include human pathogens in their intestines, while their muscle tissues are almost entirely free from microorganisms (Adams and Motarjemi, 1999). The growth of several spoilage and pathogenic bacteria are supported by an ideal substrate presented by meat and meat products. These microorganisms are not inhibited by intrinsic factors of fresh meat such as pH (5.5 —5.9) and water activity (1.00-0.98) (Mataragas *et al.*, 2008; Ellis and Good acre, 2001). Many of the microbial pathogens of current concern survive in the environment, in water, on pastures and in food, unless precautions are taken to ensure pathogen control. Contamination of raw meat with human food borne pathogens is a consequence of a wide range of pre-slaughter, slaughter and post-slaughter factors. Meat may support a mixed population of microorganisms derived from the initial animal's natural microflora, those introduced during slaughter and subsequent handling, processing and

storage (Adams and Motarjemi, 1999). Hygienic production of carcass meat is essential to ensure that contamination with potentially pathogenic bacteria is minimized (Bolder, 2007; Gill, 2007; Mead, 2004).

2.9.1 Spoilage of meat by microorganism

Food becomes a waste to humans when steps are not taken to prevent spoilage. The spoilage process occurs initially when foods are harvested or animals are slaughtered. When food becomes aesthetically unacceptable to the consumer, it's considered to be spoiled by bacteria. Bacterial spoilage in food result in a variety of sensory defects, such as off flavors, formation of slime, colour changes or strong odors (Jackson *et al.*, 1997).

2.9.2 Factors affecting spoilage

Highly perishable foods are foods that have been harvested and are processed a little further or none at all. These foods are extremely susceptible to the action of bacteria and are easily spoiled by their metabolic activities (Gram and Dalgaard, 2002). Conditions external to the food or factors inherent in the food affect the growth and selection of microorganisms in foods (Gram and Dalgaard, 2002). In order for bacterial spoilage to occur a number of factors need to be in place. These include intrinsic factors, extrinsic factors and implicit parameters. Growth of bacteria and the resultant spoilage in food are influenced by the control of the above factors (McDonald and Sun, 1999).

2.9.3 Intrinsic factors affecting microbial growth

Intrinsic factors involve properties such as availability of nutrients, acidity, water activity, oxidation-reduction potential and antimicrobial substances inherent in food (Huis in't Veld, 1996). Food needs to be suitable for the growth of the contaminating bacteria in order for spoilage to occur. The availability of nutrients in the food product

affects the selection and growth of spoilage bacteria. Meat microflora catabolizes glucose, lactic acid, certain amino acids, nucleotides, urea and water-soluble proteins present in meat (FDA, 2003). These compounds serve as essential energy sources and concentration of these compounds affect the type and rate of spoilage (Nychas *et al.*, 2008). The pH of food plays an important role in the presence and type of bacteria. The ability of food to resist changes in pH is known as its buffering capacity and foods with low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms as they grow (FDA, 2001). The activity and stability of macromolecules such as enzymes are affected by the acidity and alkalinity of an environment, the growth and metabolism of microorganisms will therefore be affected by pH (Adam and Moss, 2008). High pH in meat favors the domination of bacterial spoilage and putrefaction, while low pH meat is usually considered shelf stable and are not spoiled by microbial growth (Gram and Dalgaad, 2002).

The ability of bacteria to colonize food is affected by the presence and availability of water. The metabolic activity of microorganisms is affected by the foods water activity (a_w), since all chemical reactions of cells require an aqueous environment (Jay *et al.*, 2005). Elimination of bacterial growth occurs in food when a decrease in water activity (a_w) arises and only extremophiles and fungi are capable of development (Nychas *et al.*, 2008). Gram-negative bacteria are more sensitive to low water activity ($a_{W)}$ than Gram-positive bacteria (FDA, 2001). The oxidation-reduction potential of food affects spoilage by microorganisms. The ordered sequence of both electron and hydrogen transfer reactions is essential for the electron transport chain and energy generation by oxidative phosphorylation in living cells (Adams and Moss, 2008).

Based on their relationship to redox potential (Eh) for growth, the major groups of microorganisms include aerobes with growth at the range +500 to +300mV, anaerobes with growth at the range +100 to less than -250mV, facultative aerobes with growth at the range +300 to -100mV and microaerophilic (FDA, 2003). The metabolic activity of aerobic microorganisms decrease the Eh of food, by depleting 02 levels present, which subsequently provides anaerobes with a suitable environment in which to grow (Jay *et al.,* 2005). Most foods naturally contain antimicrobial substances which affect the growth of contaminating bacteria (Prescott *et al.*, 2002). These substances differ in their range of activities and potencies, and are present at varying concentrations in the natural food, but are frequently at levels too low to have an effect (Adams and Moss, 2008).

2.9.4 Extrinsic factors affecting microbial growth

Environmental factors such as temperature, humidity and gaseous atmosphere composition during the storage of raw meat affects the selection of certain bacteria, and affects their growth rate and activity (McDonald and Sun, 1999). Rapid temperature reduction on the carcass surface generally decreases microbial growth and therefore extends the shelf life of the product. Reduction of refrigeration temperature not only affects bacterial growth, but also the composition of the bacterial flora (Nychas *et al.,* 2008; Borch *et al.,* 1996). Bacterial growth is rapidly initiated at low temperatures once relative humidity increases and becomes high. Microbial growth thus arises when moisture absorption occurs on the food surface in moist environments (Adams and Moss, 2001). Moist atmospheric conditions favour a consortium of bacteria which are responsible for spoilage of meat stored at and between -1 and 25°C (Prescott *et al.,* 2002; Ellis and good acre, 2001). Microorganisms are affected by gases such as carbon dioxide (CO_2), ozone **(03)** and oxygen (02), as they have a direct toxic effect that may inhibit growth and proliferation (FDA, 2001). A variety of bacteria are able to grow to

high final numbers in high oxygen atmospheres, while high CO_2 atmosphere conditions inhibit the growth of Gram-negative bacteria. A modified atmosphere generally affects the structure of microbial community, shifting it from Gram-negative to Gram-positive organisms when lactobacilli begin to dominate (Prescott *et al.*, 2002; Borch *et al.*, 1996). Table 2.1 shows microbial counts on meat samples as reported by Sulley (2006). Table 2.2 shows the microbial limits for raw meat.

Table 2.1: Microbial count on meat samples and other swabs

Samples	Microbial count (cfu per gram)
Offal	1,920
Slaughter floor	1,850
Meat van	1,750
Boneless meat	960
Cutting slab	520
Clothing	68

Source: Sulley(2006)

Table 2.2: Meat Standard Committee (MSC) Microbiological limits for raw meat

Meat quality	cfu per gram
Excellent	Not detected
Good	1-10
Acceptable	10-100
Marginal	100-1000

Source: Marks (2005)

2.9.5 Causes of meat spoilage

Meat quality can be affected by the cumulative effects of chronic or continued environmental stressors (Miranda-de la Lama *et al.,* 2009; 2010). Traditionally, producers focus on the economic benefits of overloading animals. Nowadays, however, animal production is no longer focused on just economically efficient meat production, but conditions during transportation and the welfare of transported animals are becoming important (Chai *et al.,* 2010). The term stress was first used by an endocrinologist, Hans Selye who defined it as being a non-specific phenomenon representing the consequences of the behavioral, physiological and emotional status of a human or an animal to respond appropriately to a wide variety of environmental stimuli (Chai *et al.,* 2010; Terlouw, 2005). The detrimental effects of pre-slaughter handling, stunning and transportation on meat quality have been reported by several researchers (Perez *et al.*, 2002; Velarde *et al.,* 2000; Gosalvez *et al.*, 2006; Muchenje *et al.*, 2009). Chai *et al.* (2010) reported that many stimuli such as ambient temperature, humidity, noise, stocking densities, transport or lairage duration and management can influence animal welfare during the pre-slaughter period and subsequently meat quality. Other factors, besides transport time, that can induce stress on animals during road transportation and subsequently affect meat quality, are loading and unloading, stocking density and weather conditions. Vehicle characteristics (poor vehicle design increases the incidences of bruised carcasses (Dalla Costa *et al.,* 2007; Vimiso, 2010), food and water deprivation or mixing animals from different groups (Perez *et al.*, 2002; Gosalvezet *al.*, 2006), restraint, handling, and novelty of the pre -slaughter environment, adverse weather conditions, hunger, thirst and fatigue (Muchenje *et al.*, 2009), speed variations and

vibrations of the truck, contact with strangers, high stocking density and animal commingling and establishment of new hierarchies. Weather conditions such as humidity, high temperatures and air velocity (Gallo *et al.,* 2003; Mota-Rojas *et al.,* 2006). Physiological responses of animals as a consequence of transportation result in physiological stress and/or physical fatigue and can even lead to death (Mota-Rojas *et al.,* 2006). On the other hand, poor handling can cause economic losses to farmers, transporters and slaughter houses (Mota-Rojas *et al.,* 2006). In addition, injuries produced during the transportation or at lairage affect carcass temperature and pH (Gallo *et al.,* 2003; Mota-Rojas *et al.,* 2006) leading to alterations in carcass shelf-life. Meat quality depends on both animal- related and environmental factors (Lammens *et al.,* 2007) and these factors can affect muscle metabolism, thus influencing the development of PSE-meat (pale, soft and exudative), a major problem in the pork industry. However, for the consumer the surface colour of meat is the most important quality attribute at the time of purchase (Juncher et *al.,* 2001). Furthermore, season of slaughter has been shown to influence the welfare of pigs (Gosalvez *et al.,* 2006). Gregory (2010) reported that extremes in summer time temperatures increase the risk of deaths on arrival (DOAs), the risk of pale soft exudative (PSE) meat in pigs and turkeys, as well as dark cutting beef in cattle and increased concentrations of cortisol, adrenaline, nor-adrenaline and dopamine (Kadim *et al.,* 2009). The rising environmental temperatures will pose a greater risk of meat spoilage and carcass contamination with *E. coli* in poultry and Salmonella in a range of species (Gregory, 2010). In addition, cold temperatures and poor vehicle design increase the incidence of bruised carcasses (Dalla Costa *et al.,* 2007). Therefore, time of year might be a medium stressor that acts independently from transport time (Maria *et al.,* 2006).

Hence, the seasonal temperatures are said to be the main reason for differences in meat quality (Kadim *et al.*, 2008). Consumers need more transparency and information about pre-slaughter handling on experience for animals (Miranda-de la Lama *et al.*, 2010). On the other hand, farmers need to be well informed of the effects of handling on meat quality at different season of the year. For example, summer high temperatures increase incidences of dead on arrivals (DOAs), pale soft exudatives (PSE) and dark cuts. Seasons have been shown to influence the welfare of pigs while transportation of animals for long times in open trucks at high ambient temperatures may cause significant negative physiological responses in animals.

2.10 Contamination of live animal for slaughter

Live animals can be carriers of pathogenic bacteria, with high numbers of bacteria present on the skin, 'normal flora' of the skin and organisms of soil, water and fecal origin (Koutsoumanis and Sofos, 2004). There are many factors influencing the numbers and species of organisms present on the animals, including climate, geographical location, method and distance of transportation and holding conditions at the plant. For example, soil bacteria are more common on animals raised on pasture, whereas enteric origin bacteria are more common in animals raised in pens (Koutsoumanis and Sofos, 2004). On arrival at the abattoir the animals are examined. This is the first opportunity to recognize those animals that may act as a potential source of contamination, suspected of being infected by a disease and injured animals, therefore eliminating them from slaughter. If the slaughter is not done within 24 hours, the examination must be repeated (Neetling, 2005). The exterior surfaces (hide, hair, skin) of healthy live animals are

naturally contaminated with large numbers (10^7 organisms per cm^2 of hide) of a variety of organisms (Featherstone, 2003). Slaughter stock themselves are therefore a major source of carcass contamination. The hide or intestinal tracts of slaughtered animals are the main areas where potentially pathogenic and spoilage bacteria reside. The soil (ground) is also a major source of micro-organisms and has comparable numbers (10^7) of bacteria per gram of soil. Feces are about 100 times more contaminated and have an aerobic plate count and coliforms of about 10^9 and 10^8 per gram of feces, respectively (Featherstone, 2005).

The number of live animals that carry *Salmonella spp.*, are strongly correlated with the number of contaminated carcasses at the end of the slaughter5 line (Berends *et al.*, 1997), with this cross contamination estimated to account for 29% of the positive carcasses (Botteldoorn *et al.,2003).* Using *Salmonella as* an indicator organism, it has been found that the spread of this microbial contaminant, and hence other micro-organisms, is very likely to occur during transportation, where animals are in close contact with each other (via body contact) and with floors/surfaces contaminated by other infected animals (Koutsoumanis and Sofos, 2004). Research has failed to establish a relationship between visibly dirty animals and the microbial condition of the carcass; therefore, it is thought that, processing is more important than the condition of the skin (Koutsoumanis and Sofos, 2004).

The cleaning and disinfection of lairage pens has been shown to decrease the prevalence of culturable *S. enterica* in these pens, but the ability of this to reduce the prevalence in live pigs was not conclusive (Schmidt *et al.,* 2004). An alternative to the use of holding pens at abattoirs and the associated risk of the spread of *Salmonella enterica* between pigs is to hold the pigs in the transport trailers until slaughter. This has been shown to decrease the levels of infected animals entering the slaughter plant (Rostagno *et al.,*

2005). A reduction in the numbers of *Salmonella* and other micro-organisms in the intestines at pre-harvest can reduce the contamination at later stages (Beloeil *et al.*, 2004). The feeding of coarse-ground grains in comparison to fine-ground grains is known to decrease the proportion of Salmonella-positive pigs, as the coarse particles stimulate the micro biota and the production of organic acids such as lactic acid, lowering the pH in the stomach (Kim *et al.*, 2005). The inclusion of sodium chlorate in pre-slaughter feed suppresses pathogen numbers in the gut (Anderson, 2001).

The time between the last meal and slaughter does affect the fullness of the stomach, a full stomach will pose a higher risk of puncture during dressing (Borch *et al.*, 1996) and the numbers of bacteria released from the stomach/caecae are affected by feed withdrawal. Coliform numbers and *E. coli* biotype 1 numbers in the stomach were not affected by feed withdrawal (for 15 hours prior to dispatch from the piggery to the abattoir) but the holding time (holding at abattoir for an additional 0-1, 2-3 or 4-5 hours) showed a decrease in the numbers between the 0-1 and 4-5 hours (Nattress and Murray, 2000). Caecal coliforms and *E. coli* biotype 1 increased as a result of feed withdrawal, and also as a result of holding time up to 4-5 hours. This implies that in the event of the release of stomach or caecal contents onto the carcass, larger numbers of *E. coli* would be released from the caeca and fewer from the stomachs of those pigs not subject to feed withdrawal (Nattress and Murray, 2000). The prevalence of caecal lacerations was not associated with feed withdrawal time, suggesting that feed withdrawal will not increase contamination of carcasses by increasing caecal lacerations (Morrow *et al.*, 2002). Recommendations of time between last meal and slaughter range from 16 to 24 hours (Murray, 2000). There is potential for a change in the bacterial flora in the digestive tract due to feed withdrawal, with the concentration of *E. coli* biotype 1 (an indicator species), for example, increasing by one

order of magnitude with 20 hours compared with 5 hours fasting post slaughter (Nattress and Murray, 2000). This suggests that feed withdrawal may decrease the potential of nicking the GI tract. The time in lairage has been shown to affect the spread of pathogenic bacteria, with pigs known to lie down after about $1^1/_2$ hours after arrival at the slaughter plant, therefore increasing the risk of cross contamination (Warriss, 2003). Visible contamination of the live animal has little effect on the microbiological condition of the carcass (Gill and Hamer, 2004), and washing the pigs pre-slaughter has no effect on microbiological contamination of carcasses. Bolton *et al.* (2002), reported that washing the pigs pre-slaughter (power hosing at 1030 kPa, water 19°C) decreased the number of *Salmonella* on the skin of pigs, from 27% (on-farm) to 10% (after washing) incidence, although subsequent stunning and bleeding increased the incidence to 50%, so pre-slaughter washing was not considered an effective control measure.

The hide and viscera of animals entering a slaughter facility are potential source of contamination with pathogenic bacteria.

2.10.1 Hygiene of animals presented for slaughter (FAO, 2006)

- Animals presented for slaughter should be sufficiently clean so that they do not compromise hygienic slaughter and dressing.
- The conditions of holding of animals presented for slaughter should minimize cross - contamination with food-borne pathogens and facilitate efficient slaughter and dressing.
- Ante-mortem inspection should be science- and risk based as appropriate to the circumstances, and should take into account all relevant information from level of primary production.

- Relevant information from primary production where available and results of ante-mortem inspection should be utilized in process of control.

2.10.2 Hygienic dressing and handling carcass

Prevent contamination of edible portions of the carcass with soiling material from the hides, skins and pelts, and from the contents of the internal organs;

Inhibit microbial growth on the surfaces of carcasses or meat;

Eliminate any carcasses or portions of carcass that are deemed unsuitable for human consumption. (FAO, 2006)

However, two main areas of concern for informal slaughter:

- That illegally slaughtered carcasses are not being inspected by trained personnel to ensure that the meat, offered for sale to the general public, is free from disease and parasites, which could be transmitted to humans(zoonosis); and
- That there is a lack of basic health and hygiene compliance, and a negative impact of the practice on the environment.

Unsuitable stable or kraal structures: these structures do not always facilitate suitable or adequate cleaning or manure removal. This ultimately leads to increased fly breeding, soil pollution, foul odors and other health related nuisances;

Inhumane slaughtering practices: animals are often slaughtered in full view of the public, and the method of slaughter is not humane as would be in an approved abattoir;

Incorrect handling procedures: the meat handlers do not wear suitable protective clothing, carcasses are often lying on the ground (contamination and soil pollution), and meat products are not always separated and in a suitable clean container;

Unhygienic disposal of waste product: waste products are often left lying on the ground, which contributes to soil pollution, fly breeding, odour, rodent's attraction and other health hazards.

Unsuitable transportation of meat products: vehicles used for the transportation of meat products are often dirty, with the meat being stored on the floor of the vehicle, and this lead to an increased risk of contamination (Meat Safety Act, 2000).

Klinger (2004) stated that the reasons for illegal and non-inspected slaughtering of animals in developing countries included:

- The eating habits of the population: people are used to eating meat only from their own animals and trust no one else to slaughter them;
- Both Jewish and Islamic religious laws require that animals be slaughtered according to a prescribed method; and
- Illegally or home-slaughtered meat is cheaper than inspected meat.

The live animal, however, is not the only source of contamination of foods. Hazards also arise from secondary contamination due to improper handling during harvesting and other processing of raw material. Handling of food requires certain practices that ensure the safety of those who will eventually eat it. This therefore requires that the consumer is informed about the possible sources of contamination for meat intended for human consumption (Cooke, 1997; McCoubrey, 1989).

2.11 Sources of meat contamination

Research has shown that the internal tissues of healthy slaughtered animals are free of bacteria at the time of slaughter, assuming that the animals are not in a state of exhaustion (Ray, 2000). When one examines fresh meat and poultry at the retail level, varying numbers and types of microorganisms are found (Ray, 2000). Animals are often slaughtered and eviscerated on the floor because of the absence of mechanical or manual hoists. This is a major source of contamination (Adeyemo *et al.,* 2009). The abattoir environment is often filthy and waste disposal is inadequate. According to the report of Adeyemo (2002), meat safety and environmental sanitation measures at Bodija

abattoir is grossly inadequate thereby giving room for contamination and exposure of humans to disease pathogens. Animals are slaughtered and processed amidst heaps of waste materials such as bones and rumen contents accumulated from previous operations (Adeyemo, 2002). Sources of food contamination may be primary, coming directly from an infected food animal or its secretions, or excretions; or secondary, resulting from contamination in handling of food (Marriot and Gravlin, 2006).

2.11.1 Primary contamination

A food animal may be slaughtered while it is either infected with a microbial pathogen or contaminated with chemical or other residues. In some instances, this presents an occupational hazard to stockyard or abattoir workers, but more often it poses a threat to the consumer. Ante-mortem inspection reveals only a small percentage of these cases (Hubert *et al.*, 1996).

2.11.2 Secondary contamination

Secondary infection may come from infected humans or live-animal carriers of pathogens, soil, equipment, excreta and hands, nasal discharges, contaminated wounds, contaminated water, insects or feed additives. Infected humans may be the source of contamination at any point in the food chain but are most frequently implicated when preparing food for the table (Hubert *et al.*, 1996). Dressing procedures currently available cannot be relied upon to prevent or remove all of the bacterial contamination on the carcass surface. What is also important is that the skinning and evisceration steps are major sites of contamination. If these procedures are conducted in a correct manner, the degree of contamination can be reduced (Trickett, 1997., Sofos *et al.*, 1999)

Pig slaughtering is much easier than cattle or buffalo slaughtering because the carcass is not very voluminous. But it is still heavy enough to require equipment for lifting and suspending. Also, the pig skin is typically not removed because it is eaten along with

the meat. With that protective covering intact during the slaughtering, less of the meat surface is exposed and thus not subjected to easy contamination. But contamination can occur during the removal of hair from the skin, which is done by scalding the carcass in hot water tanks. Hot water exceeding the required temperature damages the skin through protein coagulation, which provokes lesions during the subsequent scraping with the knife or machine. Micro-organisms can easily intrude into such lesions. Pigs put into the scalding water, can cause enormous dirt contamination. Tremendous hygiene problems with heavy meat contamination can occur if the entire pig-slaughter operation is carried out on the ground. Lairages or holding pens at slaughterhouses are significant sources for *Salmonella* contamination of slaughter hogs. *Salmonella* contamination of the carcasses is directly correlated to prevalence of *Salmonella* in live hogs. In other words, more hogs carrying *Salmonella* prior to slaughter can lead to more *Salmonella* contaminated carcasses (Rostagno *et al.,* 2003).

2.12 Method used for killing and bleeding

Most animals have dirty skins and contain large numbers of bacteria, which will result in the knife becoming contaminated when it cuts through the skin. Bacteria enter the blood stream and spread through the body. Therefore, it is of importance to sterilize the knife at 82°C in between cuts of different animals (Meat Safety Act, 2000). The first possible contamination step in the pig slaughter process is sticking, which is a potential source of microbial contamination from contaminated equipment. This is usually not a problem if good manufacturing principles are followed. The following pig dressing processes that include scalding, dehairing, singeing, polishing are major sources of cross-contamination (Koutsoumanis and Sofos, 2004). Despite the clean appearance of the pig carcass after these processes, these carcasses may be heavily contaminated with bacteria (Gill and Bryant, 1993). Scalding, the immersion of the carcass in a tank of

water 60°C for 8 minutes) results in the destruction of most bacteria on the surface of the skin (Koutsoumanis and Sofos, 2004). However, scalding at temperatures less than 60°C results in little kill of *E. coli* and *Salmonella species* (Koutsoumanis and Sofos, 2004). Scalding can also be carried out in a vat of steam (Borch *et al.*, 1996). A time-temperature combination of 60°C for 1.4 min was required to achieve a 1 log reduction in Salmonella in scald water, which is equivalent to 65°C for 0.18 minutes (Bolton *et al.*, 2003). Gill and Jones (1995) found that a temperature of 85°C for 20 seconds reduced the total numbers of bacteria by 2 orders of magnitude, and reduced non-thermoduric spoilage bacteria from 50% to 10%. No further reduction in surviving flora numbers/composition was observed with a higher temperature or a longer time.

Dehairing, the mechanical removal of the hair by rotating drums with scraper blocks which rotate the carcass and remove the hairs, is a source of recontamination by faecal matter (Borch *et al.*, 1996). It is well known that the dehairing step has a large potential for cross contamination of carcasses (Warriner *et al.*, 2002). Dehairing equipment is a likely source of contamination of pork by mesophilic enteric pathogens (Gill and Bryant, 1993), which are removed with the scalding but are re-deposited on carcasses by dehairing equipment. One way to prevent the contamination by dehairing equipment is the use of chemical dehairing (Koutsoumanis and Sofos, 2004). The greatest reduction of skin bacterial load is achieved by singeing or flaming, with recontamination commonly occurring at the scraping/polishing step (Huis in't Veld *et al.*, 1992). Singeing (800-900°C) or flaming (1000°C) for a total of 10-15 seconds, reduces the microbial count on the skin but is dependent on the temperature/time combination used (Borch *et al.*, 1996). Reduction in microbial numbers only occurs when the skin is singed and flamed at temperatures that will produce a toasted colour to the skin (Borch

et al., 1996). If singeing and flaming only raise the surface temperature of the carcass, but does not produce a toasted colour, then it fails to reduce or eliminate the bacterial contamination on the surface of the carcass (Yu *et al.*, 1999; Borch *et al.*, 1996; Gill and Bryant, 1992). Research has shown that *Escherichia. coli* from the scraper and dry polisher became distributed on wet polisher blades, band saws and butchers' hands, even though the carcasses went through a singeing step after being dry-polished (Warriner *et al.*, 2002). The drying of the skin of the carcass can also affect the microbial population, the drying out causes a decrease in bacterial load, although the loss of carcass weight is economically undesirable (Koutsoumanis and Sofos, 2004). The spraying of chilled water during the first few hours of cooling prevents these weight losses, and can assist in the cooling process as a consequence of evaporative cooling. Normal carcass chilling procedures are rapid chilling followed by slower chilling. Under commercial conditions, the exposure of carcasses to a blast of freezing air before conventional chilling is likely to substantially improve the hygiene efficiency of the chilling process (Gill and Jones, 1992). However, care must be taken to ensure that cold-shortening does not occur as this can lead to unacceptably tough pork. Carcass cooling processes must be well controlled to contain the possibility of rapid proliferation of both pathogenic and spoilage bacteria on the meat while it remains warm (Gill and Jones, 1997). Carcasses may be contaminated during the chilling process by contact with contaminated surfaces/hands, water splashes or from the air, although the main concern during the cooling process is not new contamination, but the growth/survival of existing organisms (Koutsoumanis and Sofos, 2004).The cleaning of equipment plays a role in the spread of bacteria, if equipment is not effectively cleaned and sanitized, the potential for debris to be left behind in machinery such as handsaws, conveyor belts,

trolleys or in bins or table tops leads to contamination of carcasses (Yu *et al.*, 1999). It is known that many bacteria are susceptible to drying, therefore the cleaning and drying of equipment used in processing is an important step in improving microbiological safety of pork (Gill and Landers, 2004). Effective cleaning/disinfecting of workers hands plays an important role in reducing the potential for contamination of carcasses (Koutsoumanis and Sofos, 2004). Polishing is carried out by stainless steel scrapers or nylon brushes, and contributes to spreading the microbial population over the surface of the carcass as bacteria may become established on the brushes and scrapers (Borch *et al.*, 1996). Scraping and polishing have been reported to re-contaminate carcasses (Rivas *et al.*, 2000; Yu *et al.*, 1999; Gill and Bryant, 1993), whereas Gill and Bryant (1992) found bacterial numbers to decrease after polishing. The microbiological condition of polished carcasses can be improved by heating the carcass surface with sheets of water at 85°C (pasteurizing treatment) (Gill and Jones, 1997), although these carcasses are re-contaminated during the dressing period (Gill and Jones, 1998). Berends (1997) estimated that, after singeing, 5-15% of contamination of carcasses with *Salmonella spp*. occurred during the polishing step, 55-90% during current evisceration practices and 5-35% from further processing. The gut content is well known as a major source of carcass contamination *(Bolton et al.*, 2002). Therefore, skilled, trained operators are very important, as damage to the intestines and contamination of the skin must be avoided (Huis in't Veld *et al.*, 1992). As a consequence, evisceration is a key step in cross-contamination by *Enterobacteriaceae*, with significant increases in carcass counts on post-eviscerated carcasses (Warriner *et al.*, 2002). This concurs with the report by Rivas *et al.* (2000). One of the major ways of stopping some of this cross-contamination is by sealing off

the rectum with a plastic bag immediately after it has been freed. The enclosed rectum is then withdrawn from the body through the abdominal incision with the intestines attached. Decontamination of carcasses can be carried out by 'safe' substances such as lactic acid (Berends *et al.,* 1997). However, steam pasteurization cannot be used because it increases the deleterious effects of PSE and results in excessively pale muscles of non-PSE susceptible pigs (Gill and Jones, 1997).

2.13 Slaughter and processing

The abattoir environment and slaughtering processes play vital roles in determining the wholesomeness and safety of meat. Unhygienic practices in abattoirs and during post-process handling are associated with potential health risk to consumers due to the presence of pathogens in meat and environmental contamination (Abdullahi *et al.,* 2006). Abattoir operations generate large quantities of waste which constitute a major source of environmental pollution. Improper management of water is responsible for pollution of water bodies with an increased risk of water borne diseases in humans. Working in abattoirs can also result in occupational disease and injury.

Cattle, Pigs and Guinea fowls that are earmarked for slaughter should be deprived of feed for a period of 24hours, but clean cool water should be given. This is to ensure easiness of cleaning during evisceration. To slaughter, sever the jugular vein by the neck to ensure thorough bleeding. Severing of the neck by cutting should be by the use of a sharp knife and should be done quickly to avoid unnecessary suffering by the bird.

2. 13.1 Scalding/Dry picking

Guinea fowl feathers can be removed by scalding which is done by dipping the slaughtered bird into a bowl of hot water as soon as the bird is confirmed dead but before the carcass gets cold. This makes a period of about 6-12 minutes for easy removal of the feathers. The feathers can also be dry picked. To do this successfully, the

mouth of the bird is severed first to ensure good bleeding. A knife is then thrust through the groove in the roof of the mouth into the brain. When the brain is pierced the feathers are loosened by a convulsive movement of the muscles, this makes them easier to pick.

2.13.2 Carcass dressing

Procedures for dressing carcasses must be in place to prevent cross-contamination (e.g. from hide and fleece, sanitized equipment or surfaces, digestive tract contents spillage contaminated personal equipment or clothing, other uninspected carcasses. Dressing must be carried out immediately after slaughter and in a hygienic manner appropriate for food intended for human consumption (Kirkpatrick, 2002). The dressing techniques must minimize transfer of microorganisms to the carcass surface. Task descriptions must be documented for each dressing operation to ensure operatives carry out their tasks hygienically and consistently. Dressing operations must be supervised, and the slaughter line speeds managed to ensure hygienic operator activity. Trimming of any visible contamination must be conducted prior to final carcass inspection and using a sterile knife or other suitable means. A two knife technique must be used for all tasks that involve opening of the hide or skin. All hide and fleece cuts must be "in out" or spear cut (i.e. blade cutting away from carcass, so that transfer of micro-organisms is minimized and it is ensured that the hide and fleece do not touch the carcass with the exceptions of the initial opening at the hock. The knives used must be colour coded. The technique used to open the abdomen must minimize the possibility of cutting into the stomach and intestines (Kirkpatrick, 2002).

2.13.3 Evisceration

During evisceration, the gullet (esophagus) must be nodded and tied, the rectum bunged and the bung sealed or otherwise treated to ensure effective sealing of the

alimentary canal so as to minimize carcass contamination. The removal of the gut content of the guinea fowl after scalding is by cutting open the neck muscles close to the body leaving a flap of skin to close the hole through which the crop is removed can thereafter be removed from the body.

2.13.4 Cutting of Carcasses

After going through the process indicated above, the next step is cutting the carcass into pieces for family use or sales. This is achieved by first removing leg sinews and drawing them out from the entire limb. Then cut legs off from below the knee joint. The wings and head could be removed from the body. Finally; the carcass is cut into standard meats parts. These meat cuts can then be converted into different food dishes by frying, roasting or boiling in stew and soup (Karan, 2004).

2.13.5 Halving the carcass

The operator must ensure that the saw is sterilized at 82°C after each carcass and the sterilizing cabinet must be in a good functioning condition (Meat Safety Act, 2000).

2.13.6 Carcass washing

This step comes in after the final inspection point. The carcass is sprayed with cold water to remove all blood, visible soil, slight blood marks, bone dust and marrow (Bekker, 1998; Crouse *et al.,* 1988) before going to the cold room for chilling. It is generally recommended that only approved, uncontaminated carcasses should be washed with running water in order to remove from the carcass any bone splinters and blood which might be present thus, improving the appearance of the carcass. Bekker (1998) indicated that washing of the carcasses with cold water does not significantly influence the microbiological load on beef carcasses.

2.14 Bacterial load in the gut

Withholding feed for 24 hours before slaughter is recommended to empty the digestive tract where the heaviest and potentially dangerous load of bacteria is located (Marriot, 2004). The Food and Agricultural Organization stresses the importance of not puncturing the viscera during this stage, as this will cause contamination of the carcass with bacteria (FAO, 2005). Provided that the intestinal tract is not ruptured or punctured, evisceration can be carried out with minimal contamination of the carcass (Cohen *et al.,* 2006). Sterilization of knives is also very important during this stage. Facilities for the sterilization of the knives must be provided at this workstation (Meat Safety Act, 2000).

2.14.1Microflora

There are two types of micro-organisms of interest to the pork industry: those that cause illness (food-poisoning), and those that cause spoilage (Huis in't Veld *et al.,* 1992). Meat and meat products are responsible for a major fraction of all food-borne infections (Huis in't Veld *et al.,* 1992). The main pathogenic microflora of interest to the pig slaughter industry include *Aero monas hydrophila, Campylobacter coli/jejuni, Listeria monocytogenes, Salmonella spp., Staphylococcus aureus* and *Yersinia enterocolitica* (Borch *et al.,* 1996). *Aeromonas* and *Shewanella spp.* are facultative anaerobes, can grow at —1˚C, and are often found in vacuum-packed pork (Holley *et al.,* 2004). *Campylobacter jejuni/coli* is an important cause of enteritis in humans, although they do not grow below 30˚C have a low heat resistance and are sensitive to drying and freezing, so are not a major problem if pork is stored under normal cold storage conditions (Borch *et al.,* 1996). The pig is the most important source of *Yersinia enterocolitica* infection in humans (Nesbakken *et al.,* 1994). *Salmonella typhimurium* appears to be the most important

serotype in pigs (Huis in't Veld *et al.*, 1992) and Salmonellosis is well recognized as a major health threat to consumers of pork and pork products (Beloeil *et al.*, 2004). An estimated 15% of all salmonellosis cases in The Netherlands were associated with the consumption of pork (Berends *et al.*, 1998). Both *Salmonella* and Pseudomonads are the predominant spoilage bacteria in pork products (Liu *et al*., 2006).

A study in the Netherlands demonstrated that there was a direct relationship between the prevalence of Salmonella-positive pigs, carcasses and pork, and pork-associated Salmonellosis (Berends *et al.*, 1998). If this is something that is happening in general then a reduction in the number of positive pigs will lead to a decrease in Salmonellosis in humans In general, *Salmonella typhimurium* does not cause clinical illness in pigs (van der Gaag *et al.*, 2004). The main spoilage micro-organisms on pork are *Pseudomonas lactobacillus, Brochothrix thermosphacta, Clostridium perfringens, Aeoromonas putrifaciens* (produces hydrogen sulphide) and *Enterobacteriacea.* A complete list of genera associated with the contamination of meat and poultry can be seen in a paper by Koutsoumanis and Sofos (2004). The bacterial species that ultimately causes the spoilage of meat is dependent on a number of factors, such as the dominant species at the time of packaging, the pork pH, the storage temperature and the gaseous environment surrounding the meat in the pack.

2.15 Contamination during handling and processing

During transportation and storage, the key issue is to maintain proper refrigeration temperatures and to keep the cold-chain from breaking during steps such as loading, unloading and palletization. Pelczar *et al.* (1986) are of the opinion that: "the carcass of a healthy animal slaughtered for meat and held in a refrigerated room is likely to have only nominal surface microbiological contamination while the inner tissues are sterile". After chilling, further processing of carcasses can result in product contamination.

Contamination subsequently occurs by the introduction of micro-organisms on the meat surfaces in operations performed during cutting, processing, storage, and distribution of meat. Each new surface of meat resulting from a new cut, adds more micro-organisms to the exposed tissue. However, if the meat is kept clean by preventing contamination through dirty hands, clothing, equipment and facilities and the meat is kept cold and covered, there will be little or no contamination by micro-organisms. Pelczar *et al.* (1986) indicated that fresh meat cut from chilled carcasses have its surface contaminated with micro-organisms characteristic of the environment and the implements used to cut the meat. Generally, contamination occurs when the meat comes into contact with dirty hands, clothing, equipment and facilities (Hubbert *et al.*, 1996; Trickett, 1997). According to Marriot (1994), employees are the largest source of contamination and employees who do not follow sanitary practices, contaminate food that they touch with spoilage and pathogenic micro-organisms. Employees come in contact with these micro-organisms through work and other parts of the environment while their hands, hair, nose and mouth, harbour micro-organisms that can be transferred to food during processing, packaging, preparation and service by touching, breathing, coughing or sneezing. Therefore, in the prevention of meat contamination, personal hygiene plays an important role as there are as many as 200 different species of micro-organisms on a healthy human body (Hobbs and Roberts, 1993; Featherstone, 2003). Carcass contamination not removed by trimming or washing at slaughter is spread to newly exposed surfaces, which in turn can potentially decrease the shelf life of retail cuts and ground meat in retail meat display cases (Stivarius *et al.*, 2002., Marriot, 1994). The process of chopping and grinding enables bacteria present on the meat surface, to be distributed throughout the product (Mc Nally *et al.*, 2005). The ultimate shelf life of

ground meat depends on the bacterial level of the trimmings, sanitary conditions during processing, time and temperature of processing and storage. Heredia *et al.* (2001) explained that ground meat is an especially good growth medium because of the extensive surface area provided by the grinding and because these organisms are distributed throughout the product, whereas on the uncut meat the bacteria would be present almost entirely on the outer surfaces. The bacterial population in ground meat depends upon the bacteriological quality of the trimmings and cuts that are ground, sanitation during fabrication, type of packaging, and time and temperature of storage (Koutsoumanis *et al.,* 2006). Freshly minced meat constitutes one of the most challenging of meat products for quality assurance and public health protection. If retail mince samples show microbiological counts well in excess of 10^6 per gram it is an indication of poor quality and a potential hazard, which can markedly increase if the mince is held in ambient temperature and for these reasons the storage of unfrozen minced meat is prohibited in many countries (Bialasiewicz *et al.,* 2002). The storage life of ground beef that contains 1 million bacteria per gram is approximately 28 hours at 15.5°C. At a normal refrigerated storage temperature of approximately -1 to 3 °C, the storage life exceeds 96 hours (Marriot, 1994). Shelf life is therefore obviously influenced by the initial load of contaminating micro-organisms and there is evidence that poorly cleaned mincing equipment can contribute its quota. Minced meat, unless maintained under refrigerated conditions, rapidly deteriorate (Bialasiewicz *et al.,* 2002).

Dixon *et al.* (1991) are of the opinion that strict sanitary fabrication practices of beef carcasses can (a) reduce total bacterial counts of beef steaks, (b) reduce the percentage of typical gram-negative spoilage bacteria of steaks, and (c) reduce off-odour development of refrigerated vacuum-packaged steaks. When carcasses and cuts are

subsequently handled through the food distribution channels where they are reduced to retail cuts, they are subjected to an increasing number of micro-organisms from the cut surfaces. The contamination process by pathogenic bacteria in humans may be caused by poor hygiene conditions during processing involving sick people and animals or involving feces from infected agents. Bacteria-contaminated food may also be hazardous to public health due to the excessive growth in bacteria populations at food surface or within the food. These bacteria may come from the environment and cause toxins that develop into serious health problems on intake. Hand manipulated meat, sausages, salamis and cheese are among the most consumed product worldwide.

During the various processing operations, opportunities exist for the contamination of the carcass from the environment, the process in the plant itself, contamination via knives, equipment, the hands of workers and also by cross-contamination from carcass to carcass. Some processing operations increase contaminating microorganisms or encourage their multiplication (Kabour, 2011). As a result, the microbial population changes from mainly Gram-positive rods and micrococci on the outside of the live chicken to Gram-negative micro-organisms on the finished product Banwart, 1989; Mead, 1989; Zottola and Sasahara, 1994: Mead, 2004). Efforts should be made to prevent the build-up of contamination peaks during processing.

Rinsing of the carcasses, especially during defeathering and evisceration is therefore of great importance (Mead, 1989; Anand *et al.,* 1989). Spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin. Studies conducted over the last few years show that the sites most heavily contaminated are the neck skin and less frequently on the back and the area around the vent. Fewer organisms are found around the breast, legs and under the wings (Mead, 1989; Anand *et al.,* 1989; Zottola *et al.,* 1994; Mead, 2004). The presumable reason for the neck skin

being the most heavily contaminated is that the washings from the rest of the carcass run down the neck while the carcass hangs on the conveyor (Okonkwo *et al.*, 2008).

2.15.1 Rate of cooling of carcasses5

The main reason for chilling meat is to control the proliferation of bacteria and certain other microbes such as yeast (Strydom and Buys, 1995) and moulds on meat and to reduce the rate of deteriorative chemical changes e.g. oxidation of fats causing rancidity (James *et* a/.,2006). Further, by means of chilling the shelf life of meat is lengthened by slowing down the multiplication of organisms, which cause meat to spoil, and cause food poisoning. The rate of harmful chemical changes, such as rancidity of fats is also reduced by means of chilling (RMAA, 2004). According to Savell *et al.*, 2005, meat surface temperatures remain in the growth range for *Escherichia* and *Salmonella* flora for a considerable period and *Enterobacteriaceae* counts of chilled carcasses increase during chilling. This explains the fact that although the initial microbial contamination of meat contains both mesophilic and cold tolerant bacteria, only the latter will compete successfully at chill temperatures (Strydom and Buys, 1995).

Two methods of preserving meat by low temperatures are chilling and freezing. where, meat is stored at a temperature of 0°C to 4°C during chilling and for freezing -18°C respectively. The cold temperature slows the enzyme action and the growth and development of bacteria. Thus from the above it can be said that meat can be stored longer at freezing temperatures than at chilling temperatures. Storage times as indicated above are for meat, which has been correctly packed and sealed airtight. The meat should be stored for shorter periods if the temperature is higher than the given temperatures (SANDA, 2004).

The important criteria in beef carcass chilling include:

- Meat regulations,

- Minimize carcass mass loss,

- Avoid cold shortening of muscle,

- Minimize chilling time to improve throughput (Mallikarjunan and Mitta, 1995). The air temperature in the terminal stages of chilling shall be maintained at a value between —1 and 2 °C. That for the storage of chilled carcasses, the refrigerated room sides or quarters be maintained within the range of -1 to 5°C and the mean air speed over the product be maintained above 0.5 meters per second. The relative humidity shall be maintained below 95% and if the product is stored for longer than 72 hours, the relative humidity should be maintained below 90%.

2.16 Dispatch and transport of meat from abattoir to sale point

Maintaining the cold chain as well as hygiene during the transport of meat is of the utmost importance. Unnecessary contamination and microbiological growth will be the result if there is a breakdown of the cold chain and will have a direct impact on the shelf-life and safety of the meat. According to the Meat Safety Act, 2000 (Act 40 of 2000), the vehicle used for the transport of meat shall comply with the following in order to prevent contamination of the meat:

- The driving cab shall be completely separated from the freight compartment
- It is important that the freight compartment is in a good state of repair. The freight compartment shall be of the fully enclosed type (dustproof), continuously lined with a smooth (free from joints), easy to clean, rust free, non- toxic and non-absorbent interior surface material
- Insulated and/ or mechanically refrigerated in such a way that the temperature of the meat shall not rise more than 5°C per hour more than 2°C during the duration of local transport (less than 200km)

- For the purpose of carrying, sides or quarters, the vehicle shall be fitted with beams and stainless steel hooks in a suspended position, clear of the floor.
- No square centimeter of the said surface shall upon analysis contain more than 100 viable micro-organisms".

To further prevent contamination, the following transport practices are required:

- Carcasses, portions or red offal may not be transported in the same loading space, provided such rough offal is transported in clean water proof containers with tight fitting lids complying with specifications for equipment.
- Exposed carcasses or meat may not be transported in the same loading space as cartooned products.
- No food shall be transported simultaneously with any person or items; or in such a manner that it comes into contact with the floor or anything else that can pollute, spoil or contaminate the meat in any way.
- Conformance to good hygiene practices shall apply to workers loading, transporting and offloading meat or edible products" (Meat Safety Act, 2000).

Special care should be taken in order to prevent contamination due to the nakedness of carcasses during the unloading of meat. This area may be a major source of contamination through handling during loading and unloading and contact with vehicle surfaces (Bekker, 1998). Nychas *et a/.,* (2008) also concluded that after chilling the amount of contamination increases slightly with further increase during transportation from the packing plant to the retail store. The high levels of contamination may be attributed to more contamination through handling and changes in meat temperature during transportation (Nychas *et al.,* 2008). Vehicles for the transportation of meat and carcasses should be considered as an extension of the refrigeration process. The main objective must be to maintain the meat temperature at or near 0°C. Before loading

proceeds, the meat should be chilled to 0°C. To minimize the temperature, rise and to avoid condensation on the meat surface the temperature in these vans can be set and controlled (FAO, 2005).

2.16.1 Carcass packaging and transporting

After chilling process, the carcasses should be dried by putting them in a crate/box. The crate or box must not touch the floor directly. Do not dry the carcasses on the floor even though it is covered with plastic. Cool temperature should be maintained by adding blocks of ice to the topmost of carcass stack to avoid the growth of microorganism. Transporting carcass must be done using a closed vehicle, preferably refrigerate truck. When using an opened car, carcass should be kept inside box and the topmost part of the carcass should be covered with blocks of ice. Then, cover all outer side of the box's surface using plastic, to prevent pollution of dust during the travel (Meat Safety Act, 2000).

2.16.2 Storage and shelf life

Meat is a highly perishable product and must be stored under refrigerated conditions to control microbiological growth and other deteriorative changes. The shelf life of fresh meat is largely determined by three factors: the number of bacteria that are present on the freshly cut meat surfaces at the time of packaging; the temperature at which the it is stored; and the type of packaging material and gaseous environment surrounding the package. Shelf-life of meat is often used to describe the length of time before the product will spoil, or more specifically, the time required for spoilage organisms to reach an unacceptable level. This growth of spoilage organisms renders the product organoleptic ally undesirable but not necessarily unsafe. One of the most effective practices for improving the safety and quality of meat is proper storage temperature (Koutsoumanis and Taoukis, 2005).

To achieve storage of > 7 weeks the bacterial load on the eviscerated cooled pork carcasses must be < 2 log cfu $Cm^{-l'}$ as by 8 weeks of vacuum package storage this bacterial load (mainly lactic acid bacteria) is > 6 log cfu cm ^{1,} the maximum number for acceptance by some consumers (Holley *et al.,* 2004). The time for which meat can be stored at chill temperatures is influenced mainly by the species of animal, pH, initial level of bacterial contamination, storage temperature and the type of packaging. High pH (6.0 or higher) meat will spoil quicker than meat with a pH of 5.3 to 5.7. The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the keeping quality of the meat. So to increase meat quality assurance in accordance with microbial load assessment is deemed necessary (Yousuf *et al.,* 2008).

2.17 Types of packaging

2.17.1 Modified atmosphere packaging

The development of modified atmosphere packaging (MAP), mainly to extend shelf life of products, has resulted in increased shelf life and higher quality in response to consumer demand (Sivertsvik *et al.,* 2002). MAP involves replacing the air in a package with a fixed gas mixture, the 3 main gases used are oxygen, nitrogen and carbon dioxide, usually in combinations of 2 or 3 (Sivertsvik *et al.,* 2002). These gases have different properties, carbon dioxide inhibits the growth of bacteria and moulds, nitrogen inhibits the oxidation of fats and pack collapse, and oxygen prevents anaerobic growth (Rao and Sachindra, 2002). For products with high levels of unsaturated fats, like pork, with shelf-life limited by microbial growth and oxidative rancidity, a gas mixture of CO2 and N2 is recommended, with complete removal of 0_2. Many other gases have been tested, for example carbon monoxide, ozone, helium, ethylene oxide, but

regulations, safety concerns, reduced sensory quality or economic factors have limited their use (Sachindra, 2002).

The atmosphere in MAP changes with time. The gas composition changes with time owing to the diffusion of gases in and out of the product, the permeation of the gases in and out of the pack (no pack except aluminum foil laminated pouches exclude the diffusion of gases) and the product and microbial metabolism (Church, 1994). Also the effect of the modified atmosphere has different effects on the various types of microorganisms in the pack, for example *Pseudomonas* and *Enterobacteriaceae* are more inhibited by MAP than lactic acid bacteria (Rao and Sachindra, 2002).

2.17.2 Vacuum and CO2 packaging

Vacuum and CO2 packaging has been shown many times to reduce or inhibit the survival or growth of pathogens on meat products, a summary of this has been reviewed by Rao and Sachindra (2002). For example, CO2 has an inhibitory effect on Salmonella, and the degree of inhibition is increased as the storage temperature decreases. Lactobacilli replace spoilage organisms in MAP fresh meat as they are less sensitive to CO_2 (Rao and Sachindra, 2002). For retails cuts of meat, if the time between meat cutting and display is short, then simple over-wrapped trays are used (Gill and Jones, 1997). If longer storage is desired, it is necessary to use modified atmospheres and if longer storage times are required then vacuum or CO2 storage is best (Gill and Jones, 1997). This same study showed that if chops were stored under either N2 or CO2 then their appearance was similar to fresh pork chops after 42 days of storage. Moreover, chops stored under vacuum or CO_2 for 42 days showed no objectionable odour, whereas those stored under N2 for 28 days or longer, or 02+CO_2 for 21 days or longer had stale sour odour. The results of the study indicated that the storage time of pork chops under N2 or

0_2+CO2 was around a week, and was >3 weeks if stored under CO2 (Gill and Jones, 1996). Other studies have shown that storage lives of > 8 weeks are feasible so long as the hygiene in the cutting room is good, the meat is packaged in CO2 packs and the stored packs are held at -1.5°C (Holley *et al.*, 2004). MAP and Value Package. (VP) products are 'safe' so long as they are held at correct chill storage temperatures (< 4°C) (Rao and Sachindra, 2002), whereas under inadequate storage conditions both *Clostridium botulinum* and *C. perfringens* could grow and produce toxins, causing food poisoning.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was carried out in fifteen selected retail meat shops for beef, pork and Guinea fowl meat in Bolgatanga. Bolgatanga is the capital town of the Upper East Region of Ghana. The ethnic group is the Grunsi tribe with their language being Grune language spoken also called Frafra (GSS, 2002). The vegetation is primarily savanna grassland. The climate is very dry and is characterized by one rainy season from May/June to September/October. The mean annual rainfall during this period is between 800 mm and 1.100 mm. The economic activity of the people is mainly subsistence agriculture.

3.2 Sources of meat samples/Sampling Procedure

Fifteen retail meat shops were selected in the Bolgatanga Municipality where most people prefer to buy beef, pork and guinea fowl meat for this study. The retail shops were Stanbic, Star life, Central mosque, Jolly hut, and Mobile clinic (for beef); Soe, Atulbabisi, Pobaga, Zobisi, and Dagbew (for pork); Atibire, Next door, Comeci, Speed link and Ojam (for guinea fowl). A total of 150 raw fresh and smoked meat samples.Ten (10) meat (5 fresh and 5 smoked) samples were collected from each retail shop. An area of 10 cm2 was swabbed and swabs transported under 4oC to the University for Development Studies (UDS) laboratory for microbial analysis. Some of the bacteria enumerated were identified using Gram staining and catalase test. The experiment was carried out between the periods of April 2013 to June 2014.

3.3 Preparation of sample

This was done according to Adzitey *et al.* (2014). Swabs were placed in 10 ml sterile peptone water and thoroughly shanked to obtain the neat (10-1). One (1) ml of the neat was transferred into 9 ml sterile peptone water until a dilution of 10-6 was obtained. Serial dilutions (10-1 to 10-6) were spread plated onto blood and nutrient agar plates.

3.4 Culturing of bacteria

The media containing the inoculums were incubated in an incubator at a temperature of 37°C for 24 hours under aerobic condition and the colony forming units(30-300cfu/cm^2) were counted to obtain the microbial load. Colony forming unit was calculated using the formula:

$N = \sum C / [(1 * n1) + (0.1 * n^2] * (d)$

Where N = Number of colonies per cm^2

$\sum C$ = Sum of all colonies on all plates counted

n1 = Number of plates in first dilution counted

n^2 = **Number** of plates in second dilution counted

d = Dilution from which the first counts were obtained (Maturin and Peeler, 2001).

3.5 Identification of microbes

3.5.1 Gram staining

After culturing, the morphology and appearance of some colonies were studied using Gram staining technique. The gram staining technique was also used to determine whether the bacteria are Gram negative or Gram positive. In this technique, a sterile wire loop was use to pick a colony from the media plate, spread over a glass slide and air dried for about 10 minutes. After which four stages of staining process (flooding and washing) was undertaken as follows:

- Crystal Violet (for 30 seconds)
- Acetone Alcohol (decolorized) (rapidly)
- Red counter stain (safranine) (for 1 minute)

After staining, the slide was left to dry for 30 minutes and was placed under a microscope (x100 oil immersion lens) to differentiate between Gram positive and Gram negative bacteria and to identify specific bacteria based on their morphology (Bacteria under Microscope, 2013).

3.5.2 Catalase test

The Gram staining was followed by catalase test. This was used to test the ability of bacteria to degrade hydrogen peroxide **(H202).** The production of bubbles after the addition of the hydrogen peroxide indicates that catalase is present. Catalase test was performed using a sterile wire loop to pick a colony from the blood agar plate and placed into a test tube containing about 0.5ml of hydrogen peroxide. A bubble formed around the loop indicated catalase positive for the test, whilst absent of bubble indicated a negative catalase test.

3.5.3 Visual assessment

Various poor handling and unhygienic practices were observed during data collection. For instance, it was observed that butchers handling meat paid little or no attention to their personal hygiene and served the meat with dirty hands and clothing. Meats were put on tables which are not well cleaned before and after the day's work and also in the open exposing the meat to houseflies.

Poor sanitation was also observed in the immediate environment were meats are sold. Adzitey *et al.* (2014) observed similar unhygienic practices in the handling of meat in the Yendi Municipality of the Northern Region of Ghana.

3.5.4 Data analysis

All data collected was analyzed using Analysis of Variance (ANOVA) of the Genstat Statistical Package, 6th Edition.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

The result obtained from sampling beef is shown in Table 4.1. The total aerobic count for beef ranged from 4.75 to 6.56 log cfu/g. There were no significant differences ($P > 0.05$) among the fresh and smoked beef samples collected from the five `different beef sale points. In absolute terms, fresh beef samples from Jolly hut exhibited the highest total aerobic count of 6.56 log cfu/g and fresh beef from Star life exhibited the lowest total aerobic count of 4.75 log cfu/g. It can also be observed that about 80% of the samples had total aerobic count of more than 5.00 log cfu/g which is an indication of high meat contamination for the beef samples, however, none of the beef samples were spoiled since the mean viable count of the beef samples did not exceed 7.0 log cfu/g. Warriss (2001) reported that when the microbial load of meat samples is 10^6 log cfu/cm^2, spoilage is eminent.

Figures below show box plots of beef, pork and guinea fowl products by comparing fresh and smoked meat

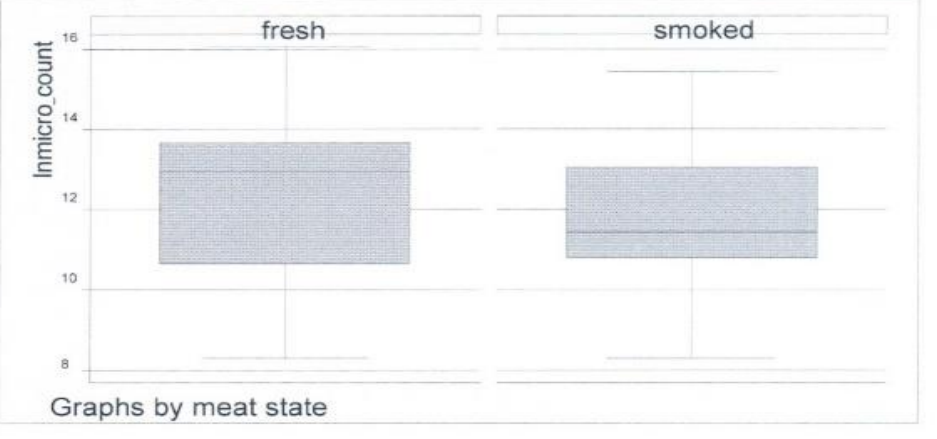

Figure 4.1: Box plot of log microbes' count beef

Fresh beef seems to the high median of log microbes count as compare to smoked beef, the median of fresh beef appear to be close to the upper quartile while the smoked median is also closed to lower quartile.

Table 4.1 : Total count of beef

Area/Type of Beef	Aerobic Plate Count (cfu/g)	log (cfu/g)
Stanbic Fresh	2.03×10^6	6.31
Stanbic Smoked	1.00×10^5	5.00
Star Life Fresh	5.68×10^4	4.75
Star Life Smoked	2.04×10^5	5.31
Central Mosque Fresh	3.32×10^6	6.52
Central Mosque Smoked	1.31×10^6	6.12
Jolly Hut Fresh	3.64×10^6	6.56
Jolly Hut Smoked	2.00×10^5	5.30
Mobile Clinic Fresh	1.52×10^5	5.18
Mobile Clinic Smoked	3.17×10^5	5.50
Sed	1418586	
P-value	0.074	

Sed = Standard error of difference

The result obtained for fresh and smoked pork is shown in Table 4.2. It can be seen that the total aerobic count for pork ranged from 4.33 to 6.94 log cfu/g with smoked pork from Zobisi having the highest total aerobic count of 6.94 log cfu/g and fresh pork from Soe having the lowest total aerobic count of 4.33 log cfu/g. Significant differences ($P < 0.05$) existed among the fresh and smoked pork samples obtained from the five different pork sale points.

Smoked pork samples from Zobisi and Pobaga were significantly higher ($P < 0.05$) than the rest of the fresh and smoked pork samples examined. It is unusual for smoked meat samples to be higher in microbial load than fresh meat samples. It was expected that the heat the meats were exposed to will reduce the microbial load. Nonetheless, the high microbial load could be due to cross contamination after smoking. From Table 4.2, it can also be observed that about 70% of the pork samples obtained had total aerobic counts of more than 5.00 log cfu/g which indicates high meat contamination, however, none of the pork samples were spoiled because the mean viable count of the pork samples was below 7.00 log cfu/g.

Figure 4.2: Box plot of log microbes' count pork

For pork median for both fresh and smoked all appear to be in the middle which the pork data seems to be normally distributed but smoked pork had a higher median than fresh pork. Difference between median log microbes count may not be substantive different from each other.

Table 4.2 : Total count of pork

Area/Type of Pork	Aerobic Plate Count (cfu/g)	log (cfu/g)
Zobisi Fresh	$1.39x10^{5b}$	5.14
Zobisi Smoked	$8.66x10^{6a}$	6.94
Pobaga Fresh	$1.00x10^{5b}$	5.00
Pobaga Smoked	$1.09x10^{6a}$	6.04
Soe Fresh	$2.13x10^{4b}$	4.33
Soe Smoked	$7.02x10^{4b}$	4.85
Atulbabisi Fresh	$2.33x10^{5b}$	5.37
Atulbabisi Smoked	$1.77x10^{5b}$	5.25
Dagbew Fresh	$6.32x10^{5b}$	5.80
Dagbew Smoked	$1.77x10^{5b}$	5.25
Sed	2290967	
P-value	0.015	

Sed = Standard error of difference;

Means in the same column with different superscript are significantly different.

The results obtained from the guinea fowl meat is shown in Table 4.3. The total aerobic count ranged from 4.90 to 6.73 log cfu/g with smoked guinea fowl meat from Next Door having the highest total aerobic count of 6.73 log cfu/g. On the contrary, smoked guinea fowl meat from Atibere had the lowest total aerobic count of 4.90 log cfu/g.

Box plot of fresh and smoked guinea fowl meat is shown below;

Figure 4.3: Box plot of log microbes' count guinea fowl

It is quite apparent that median when the data is normally distributed will be the same as the mean can be used to show that log of mean of microbes count of guinea fowl meat might be equal or not. As we can see there seems to be some difference between the two means which we have to confirm with the t test statistic.

Table 4.3: Total count of guinea fowl meat.

Area/Type of Guinea Fowl	Aerobic Plate Count (cfu/g)	log (cfu/g)
Next Door Fresh	$7.08x10^{5bc}$	5.85
Next Door Smoked	$5.39x10^{6a}$	6.73
Comeci Fresh	$1.14x10^{5c}$	5.06
Comeci Smoked	$2.78x10^{6b}$	6.44
Speed Link Fresh	$1.73x10^{6c}$	5.24
Speed Link Smoked	$7.88x10^{5bc}$	5.90
Atibere Fresh	$2.79x10^{5c}$	5.45
Atibere Smoked	$7.96x10^{4c}$	4.90
Ojam Fresh	$3.76x10^{5c}$	5.57
Ojam Smoked	$2.81x10^{5c}$	5.45
Sed	1108023	
P-value	<.001	

Sed = Standard error of difference;

Means in the same column with different superscript are significantly different.

Significant differences ($P < 0.001$) existed among the fresh and smoked guinea fowl samples obtained from the five different guinea fowl meat sale points. Smoked guinea fowl meat from Next Door was significantly higher ($P < 0.001$) than the rest of the guinea fowl meat samples. Smoked guinea fowl meat collected from Comeci was also significantly higher ($P < 0.001$) than the rest except fresh meat from Next Door and smoked meat from Speed Linked. The high microbial contamination of the smoked guinea fowl meat might have resulted from cross contamination of the meat after

smoking. From Table 4.3 about 90% of the samples obtained had a total aerobic count of more than 5.00 log cfu/g which is an indication of high meat contamination; however, are in the cases of beef and pork, the guinea fowl samples obtained were not spoiled since none had a total aerobic count of 7.0 log cfu/g and above.

In this study more than 50% of the data obtained showed that the fresh meat in the case of the pork and guinea fowl had lower total aerobic microbial count than their smoked counterparts. For example, in Pobaga the microbial load for fresh pork was 5.00 log cfu/g and that for the smoked counterpart was 6.04 log cfu/g, the situation was not different for fresh guinea fowl meat from Comeci which showed a load of 5.06 log cfu/g and the smoked showed a load of 6.44 log cfu/g. However, the situation was quite different in the case of the beef where the fresh meat generally recorded higher aerobic plate counts than their smoked counterparts. For instance, fresh beef from Stanbic had a load of 6.31 log cfu/g whereas the smoked one was 5.00 log cfu/g. Also fresh beef from Jolly hut recorded a load of 6.56 log cfu/g and the smoked one was 5.30 log cfu/g are shown in Table 4.1.

The highest microbial load for the fresh meat samples was fresh beef (6.56 log cfu/g) from Jolly hut whereas the highest microbial load for the smoked meat samples was pork (6.94 log cfu/g) from Zobisi. The lowest microbial load for the fresh and smoked meat samples were 4.33 log cfu/g, 4.85 log cfu/g respectively both from Soe. The differences in the load can be attributed to the way the meats were handled. Poor handling and unhygienic practices were observed during data collection. For instance, it was observed that butchers from retail shop handling meat paid little or no attention to their personal hygiene and served the meat with dirty hands and clothing. Meats were put on tables which are not well cleaned before and after the day's work and also in the open exposing the meat to houseflies. Poor sanitation was also observed in the immediate surroundings where meats are sold. Adzitey *et al.* (2014) observed similar unhygienic practices in the handling of meat in the Yendi Municipality of the Northern Region of Ghana. The afore-mentioned practices contributed to the high microbial load and the differences in the microbial observed.

Other factors also contributed to high microbial load. Mukhopadhyay *et al* (2009) reported that, hot and humid climate contribute to increasing total aerobic counts on meat, and that could have contributed to the high total aerobic counts on the meat in this study since Bolgatanga is a hot and humid area. Under poor processing conditions pathogenic and non-pathogenic microorganisms are introduced on the meat. In addition, the high nutritional value of meat makes it susceptible to high levels of microbial contaminations. In this study 73% of the samples had more than 5.0 log cfu/g which indicates high meat contamination. High levels of microbial presence on meat increase the chances of the meat getting spoiled within the shortest possible time. Although microbial load on the meat samples were high, they were below the threshold of 7.0 log cfu/g. This is the required level for meat spoilage to occur (Warris, 2001).

The level of aerobic plate count in this study is in accordance with studies by other researchers (Alvarez-Astorga *et al*., 2002; Bhandare *et al*., 2007; Haque *et al*., 2008; Hassan *et al*., 2010).

Guideline Counts:

Products counts obtained using methods set out in these guidelines signal weather microbiological conditions are within the normal range. The following description are used: Excellent, Good, Acceptable and Marginal for microbial levels listed below:

Table 4.4: Meat Standard Committee (MSC) microbiological limits for raw meat

Category	TVC/cm or /g	cfu per gram
Excellent	<1,000	Not detected
Good	1,000-10,000	1-10
Acceptable	10,000-100,000	10-100
Marginal (Action required)	100,000-1,000,000	100-1,000

Source: Marks (2005):

Tables 4.5, 4.6, and 4.7 show the genera of bacteria identified in the smoked and fresh beef, pork and guinea fowl meat. Five different bacteria genera namely *Staphylococcus spp., Streptococcus spp., Salmonella spp., Klebsiella spp.* and *Escherichia coli* were identified in the beef samples.(Table 4.5)

Table 4.5: The genera of bacteria identified from fresh and smoked Beef.

Source	Fresh Beef	Smoke Beef
Stanbic	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Klebsiella spp.*	*Streptococcus spp., Staphylococcus spp., Salmonella spp., Escherichia coli*
Star-Life	*Staphylococcus spp., Streptococcus spp., Escherichia coli*	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli*
Central-Mosque	*Staphylococcus spp., Salmonella spp., Streptococcus spp.*	*Staphylococcus spp., Salmonella spp., Streptococcus spp.*
Jolly-Hut	*Salmonella spp., Escherichia coli*	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli*
Mobile-Clinic	*Salmonella spp., Streptococcus spp., Klebsiella spp.*	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli*

Also five different bacteria genera were identified in pork samples (Table 4.6) and these were *Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli* and *Klebsiella spp.*

Table 4.6: The genera of bacteria identified from fresh and smoked pork

Source	Fresh Pork	Smoke Pork
Zobisi	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli*	*Streptococcus spp., Klebsiella spp., Escherichia coli*
Pobaga	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli*	*Klebsiella spp., Salmonella spp., Escherichia coli*
Soe	*Klebsiella spp., Escherichia coli*	*Salmonella spp., Staphylococcus spp., Escherichia coli*
Atulbabisi	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli*	*Salmonella spp., Staphylococcus spp., Escherichia coli*
Dagbew	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Escherichia coli*	*Salmonella spp., Staphylococcus spp., Escherichia coli*

On the other hand seven different bacterial isolates were identified in fresh and smoked guinea fowl meats. These are *Staphylococcus spp., Streptococcus spp., Salmonella spp., Proteus spp., Pseudomonas spp., Escherichia coli* and *Bacillus spp.* (Table 4.7) .The guinea fowl was by far the one with the highest number of different bacterial isolates. The presence of different bacteria genera in the meat samples is an indication of the poor slaughtering and handling of animals and carcasses, and the poor environmental conditions under which meat is sold. Bacteria isolates are easily transferred to the meat due to close contact and continuous turning of meat during cutting (FAO, 1991).

Table 4.7: The genera of bacteria identified from fresh and smoked Guinea fowl

Source	Fresh Guinea fowl meat	Smoke Guinea fowl meat
Ojam	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Bacillus spp.*	*Staphylococcus spp., Streptococcus pp., Salmonella spp., Bacillus spp.*
Next-Door	*Staphylococcus spp., Streptococcus spp., Salmonella spp., Bacillus spp.*	*Proteus spp., Staphylococcus spp., Salmonella spp., Escherichia coli., Bacillus spp.*
Comeci	*Pseudomonas spp., Staphylococcus spp., Escherichia coli, Salmonella spp.*	*Pseudomonas spp., Staphylococcus spp., Bacillus spp.*
Speed-Link	*Staphylococcus spp., Bacillus spp., Salmonella spp., Escherichia coli*	*Pseudomonas spp., Staphylococcus spp., Bacillus spp.*
Atibere	*Escherichia coli, Staphylococcus spp., Pseudomonas spp., Salmonella spp., Bacillus spp.*	*Staphylococcus spp., Proteus spp., Bacillus spp., Salmonella spp., Escherichia coli.*

Overall, eight bacterial isolates were identified during the study and they were *Streptococcus spp.*,*Proteu sspp.*, *Staphylococcus spp.*, *Salmonella spp.*, *Klebsiella spp.*, *Bacillus spp.*, *Pseudomonas spp.* and *Escherichia coli* (Tables 4.5, 4.6 and 4.7). The identification of organisms such as *Escherichia coli* and *Salmonella spp.*, which are important food borne pathogens is of public health concern and therefore consumers of

meat in and around the Bolgatanga municipality need to take caution since they are at risk of food borne infection. Adequate cooking of the fresh and/or smoked meat is required in order to kill all pathogens.

Staphylococcus spp., runs through most of the samples obtained and this can be due to the contamination from the skin of the animal or humans. This is in agreement with the report by Postage (2000) that *Staphylococcus spp.*, can be part of the normal flora on the skin of humans and animals which can be transmitted from person to meats and meat products through unhygienic practices. *Staphylococcus spp.*, cause infections such as arthritis, black pox, boil, bronchitis, carbuncle, cystitis, endocarditis, meningitis, osteomyelitis, pneumonia, and scalded skin (Stuart, 2005). Sulley (2006) reported that the vehicles and trucks for transporting carcasses are inadequate, compelling others to use motor-bikes and bicycles as a means of transport. These means of transport are not properly cleaned and thus contained high microbial load. Bhandare *et al.* (2007) reported that the unhygienic practices of meat processing in developing countries results in these meats being contaminated with microorganisms. Meat sellers were also observed busily conversing, coughing, and sneezing which might result in contamination through introduction of saliva on the meat. Okonkwo *et al.* (2008) stated that, food can be infected with microorganisms as a result of coughing and sneezing from those who handle and process these foods. Koffi-Nevry *et al.* (2011) also stated that, careless sneezing and coughing among butchers can lead to contamination of the products.

The differences in microbial load of fresh and smoked meat samples can be attributed to the way the meats were handled. Poor handling and unhygienic practices were observed during data collection. For instance, it was observed that butchers from retail shop handling meat paid little or no attention to their personal hygiene and served the meat with dirty hands and clothing. The high microbial load could be due to cross contamination after smoking. Meats were put on tables which are not well cleaned before and after the day's work and also in the open exposing the meat to houseflies. Poor sanitation was also observed in the immediate surroundings where meats are sold. Adzitey *et al.* (2014) observed similar unhygienic practices in the handling of meat in the Yendi Municipality of the Northern Region of Ghana. The afore-mentioned practices contributed to the high microbial load and the differences in the microbial observed. The genera of bacteria identified in this study also included many species which are non-pathogenic *(Staphylococcus, Streptococcus),* and form part of the commensal human microbiome of the mouth, skin, intestine, and upper respiratory tract (Adams and Moss, 2008; Adzitey *et al.,* 2014). However, some species identified can be pathogenic or cause food spoilage. *Escherichia coli* is an enteric microorganism that is potentially pathogenic especially when they change their habitat (Basavarajappa *et al.,* 2005; Igumbor *et al.,* 2007). *Escherichia coli* can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and pneumonia (Guentzel, 1996; Jay, 2000; Adams and Moss, 2008). *Proteus spp.* Includes pathogen responsible for wound and many human urinary tract infections (Guentzel, 1996). *Streptococcuss spp.* can cause septic sore throat, scarlet

fever, septicemia infections, meningitis, endocarditis, erysipelas and necrouLing fasciitis (FDA, 2013). Microorganism isolated from fresh and smoked meat samples in this study have been earlier found in foods, environment and other places and their pattern is similar to previous reports by Clarence *et al.,* 2009. The presence of these organisms in fresh and smoked meat depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering, processing and packaging of meats. Faecal coliforms as Escherichia coli are generally considered as indisputable indicator of faecal contamination from warm blooded animals.

The presence of *E coli* and *Enterobacter spp* in this meat samples is an indication of faecal contamination of the meats. The differences of microbial load of fresh and smoked meat in the retail shops, for instance fresh beef samples from jolly hut exhibited high total aerobic count of 6.56 log cfu/g as its smoked counterpart recorded 5.30 log cfu/g. Smoked pork samples from Zobisi were significantly higher (6.94 log cfu/g) than the fresh pork samples examined (5.14 log cfu/g). Smoked guinea fowl meat from next door had the highest total aerobic count of 6.73 log cfu/g than fresh meat samples (5.85 log cfu/g) This might be due to possible unhygienic handling of the meats during slaughtering and processing or due to possible contamination from the skin, mouth or nose of the handlers which might be introduced directly into the meat (Schroender *et al.,* 2005). The isolation of *Enterobacter spp,* may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering (Talaro and Talaro, 2006). *Salmonella spp* is another organism found in the meats is also a pathogenic organism of public health significance and concerns

(Okonkwo et al., 2009). *E. coli* is a normal flora of the human and animal intestine and has been identified as a leading cause of food borne illness all over the world (Hussein, 2007) *E. coli* 0157.H7 strain was not detected in any of the meat samples examined. However, diarrhea caused by enterotoxigenic *E. coli* (ETEC) is highly prevalent in young children in developing countries as well as) travelers (Duffy, 2006). Fresh and smoked meat sold to the public in open retail shops are grossly contaminated with coliform bacteria as well as other bacterial forms. The findings of this study revealed that fresh and smoked meats sold in Bolgatanga municipality are contaminated with pathogenic gram negative bacteria. The possible sources of these contaminants are due to the unhygienic manner of handling meat in the retail shops. This implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The microbial assessment of the retail meat products viz., beef, pork, and guinea fowl meat in the Bolgatanga Municipality revealed the following general observations.

- In terms of fresh meats, fresh beef showed the highest microbial counts while fresh pork showed the lowest.
- In terms of smoked meats, smoked guinea fowl meat showed the highest microbial counts while smoked beef showed the lowest.
- Microbial quality of pork products was found to be better in comparison with beef and guinea fowl meat products.
    - Consumers of meat in and around the Bolgatanga Municipality need to take caution since they are at risk of foodborne infection

5.2. Recommendations

For improved hygienic meat handling the following recommendation are being made:

- Meat handlers and sellers should be educated on the adverse effects of the lack of proper personal and environmental hygiene and sanitation
- Good manufacturing practices should be strictly adhered to by butchers and those selling the meat. The equipment must be washed properly before use
- . Adequate cooking of the fresh and/or smoked meat is required in order to kill all pathogens.

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