UNIVERSITY FOR DEVELOPMENT STUDIES

EFFECT OF POST-SLAUGHTER HANDLING ON MICROBIAL AND AESTHETIC QUALITIES OF FRESH BEEF IN THE TECHIMAN MUNICIPALITY

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DECLARATION

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. All assistance towards the production of this work and all the references contained herein have been duly acknowledged.

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ABSTRACT

Salmonella and *Escherichia coli* are the two most important food-borne pathogens of public health interest incriminated in meat worldwide. These bacteria are widely distributed in food and the environment. This study was conducted to determine the prevalence of *Salmonella* and *E. coli* in fresh beef and related samples obtained from 15 beef retail points in Techiman Municipality. Consumers' attitudes towards fresh beef colour as its aesthetic quality in display was also determined. A total of 240 beef and related samples (60 each of beef, table, knife and apron) as well as 240 beef consumers were surveyed from April 2014 to November 2014. The samples were analysed using a modified method of Bacteriological Analytical Manual (BAM) of the Food and Drug Administration (FDA) whiles beef colour preference was done through photographs and hedonic assessment. Overall prevalence of *Salmonella* was 57.1% and 79.20% as overall prevalence of *E coli*. Fresh beef and table samples recorded equal levels of incidence of *E. coli*, 91.70% as the highest, knife and apron had the least prevalence 48.30%. Beef sample recorded the highest prevalence of *Salmonella* 70% and apron the least 33.3%. Hygienic practices were not strictly followed by butchers at the retail markets; therefore the beef supplied to the public are contaminated with *Salmonella* and *E. coli*. A highly significant difference ($P<0.001$) of consumer preferences for all the fresh beef colour samples were observed except samples crimson and red. Colour influences the purchasing intent of consumers more than any other quality factor as portrayed by this study. Consumers therefore use colouration as an indicator of freshness and wholesomeness.

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DEDICATION

I dedicate this work to God Almighty, my creator through Him all things are possible. Secondly to my entire family most especially my Mum Martha Anane and Dad of blessed memory, my lovely wife Janet Owusu and children Elizabeth

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LIST OF ACRONYMS

AOAC: Association of Analytical Chemist.

A_w-: Water Activity

BGA: Brilliant Green Agar

BSI: British Standard Institution

CDC: Centre for Disease Control and Prevention.

DAEC: Diffusely Adherent *Escherichia coli*

DFD: Dark Firm and Dry

EFSA: European Food Safety Authority.

EHEC: Enterohemorrhagic *Escherichia coli*

EIEC: Enteroinvasive *Escherichia coli*

ELISA: Enzyme-Linked Immuno Sorbent Assays.

EPEC: Enteropathogenic *Escherichia coli*

ESR: Environmental Science and Research.

ETEC: Enterotoxigenic *Escherichia coli*

FACS: Food Advisory Consumer Service.

FCDA: Foodstuffs, Cosmetics and Disinfectants Act

FOA: Food and Agriculture Organisation

FSANZ: Food Standards Australia New Zealand

FSIS: Food Safety and Inspection Service

GDP: Gross Domestic Product

GSS: Ghana Statistical Service

HACCP: Hazard Analysis and Critical Control Point

HC: Hemorrhagic Colitis

HCL: Hydrochloric Acid

HUS: Hemolytic Uremic Syndrome

ICMSF: International Commission on Microbiological Specifications for Food

IFAD: International Fund for Agricultural Development

IFT: Institute of Food Technologist

LEMB: Levine's Eosine Methyelene Blue

LIA: Lysine Iron Agar.

MAP: Modified Atmosphere Packaging

DMb: Deoxymyoglobin

Mb02: Oxymyoglobin

MBG: Meat Buy's Guide

MethMb: Methemoglobin

MetMb: Metmyoglobin

ML: Milliliters.

MSA: Meat Safety Act

NA: Nutrient Agar.

NDA: National Department of Agriculture

NHA: National Health Act

NNDSS: National Notifiable Disease Surveillance System

PHV: Public Health Veterinarian

PSE: Pale Soft Exudative

RMAA: Red Meat Abattoir Association

RV: Rappaport Vassilidis.

SAMIC: South African Meat Industry Company

SC: Selinite Cystine broth.

SRID: Statistics, Research and Information Directorate

TMA: Techiman Municipal Assembly

TSI: Tripple Sugar Iron

UP: Thrombotic Thrombocytopenic Purpura

VP: Voges- proskauer.

WASH: West African Shorthorn

WHO: World Health Organisation.

XLD: Xylose Lysine Deoxycholate.

CHAPTER ONE

1.1 INTRODUCTION

Meat is an edible flesh or muscle of animals that is used as food by man. These animals include cattle, sheep, goat, pigs, camels and poultry. Meat is a high protein food which is widely consumed by majority of the populace (Sutherland *et al.,* 1986). It is a very delicate product and susceptible to microbial invasion and subsequent deterioration due to its sufficient nutrients content which supports growth of microorganisms (Warriss, 2010). Most meat have high water content (70-79%) corresponding to a water activity approximately (a_w) 0.99 which is suitable for microbial growth (Warriss, 2010; Rao *et al.,* 2009). Food borne infections are major international health problem with consequent economic losses. They are also a major cause of illness and death worldwide (Adak *et al.,* 2005). Food borne infection leads to the death of many children and affects their growth and cognitive development and heavily affects health care systems (Adak *et al.,* 2005).

Bacterial activity as a major factor in pigment changes in fresh prepackaged meat has been well established when the meat is putrifying (Butler *et al.,* 1953; Costilow *et al.,* 1955).

Microorganisms grow on meat causing visual (colour), textural and organoleptic change when they release metabolites (Jackson, 2001). Colour of fresh beef during retail display is an important factor used by consumers to judge freshness of the product and make their purchase decision. A bright, cherry-red muscle

tissue colour is desired. Consumers have a keen eye for recognizing when beef colour does not meet their ideal mental image (Schaefer, 2007).

In a healthy animal, the carcass tissues and the body cavities are effectively microbiologically sterile (Romans *et al.*, 1994; Warriss, 2010). However, during slaughtering, dressing and cutting, microorganisms mainly from the exterior of the animal, its gut, the environment, handlers and equipment in general contaminate the meat (Warriss, 2010). External contamination of meat is a possibility from the moment of bleeding until consumption (Lawrie, 1991). *Escherichia coli and Salmonella* are widely distributed in the environment through contaminated food and water as the major sources of spread (Clarence *et al.*, 2009). *E. coli are* used as surrogate indicator in food as faecal contamination (Clarence *et al.*, 2009). *E. coli* strains capable of producing shiga toxins can be isolated readily from meat, poultry, and sea foods (Samadpour *et al.*, 1994). Post-mortem handling involves all the activities and processes carcasses are subjected to after sticking (Adzitey, 2011). This in turn would have effect on meat quality (Warriss, 2010). Experts and stakeholders must encourage the development of a science- and risk-based food safety system; prioritise hazards and use the best intercession available data on the distribution and reduction of risks (Batz *et al.*, 2005). This requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this continuum (Batz *et al.*, 2005). Therefore increasing meat quality assurance in accordance with prevalence of microorganisms and colour assessment are crucial (Yousuf *et al.*, 2008).

In Ghana, due to inadequate education, unavailability of potable water and reliable power supply, inappropriate technologies for processing, transporting, handling, storage of fresh beef and inadequate knowledge in post-slaughter management, meat processing is traditionally done in unhygienic conditions. Slaughter methods are sometimes dictated by religious beliefs and local customs without inspection by qualified veterinary officer, resulting in high post-slaughter contamination and losses of aesthetic value (Adzitey *et al.*, 2011).

Foods (meat inclusive) have been reported to have high incidence of bacteria in Ghana (Adoma; 2010). Adzitey *et al.* (2011) established that high bacteria count and diversity of bacterial isolates from beef is an indication of its low bacteriological quality, and this could be a potential source of food infection. Poor hygienic practices by the butchers are probable contributors to the microbial contamination on the beef. There is however limited information on the meat industry in the Brong Ahafo Region on the prevalence of *E. coli* and *Salmonella* in fresh beef at the slaughter and retail points within a highly populated community. It is therefore, vital to give useful information on the incidence of *Salmonella* and *E. coli* on fresh beef sold in Techiman. Moreover there is inadequate knowledge of beef handlers of the effect of post slaughter handling on the aesthetic value which is the driving force on consumers' decision of purchasing. This study therefore, focuses on prevalence of *E. coli and Salmonella* in fresh beef sold in Techiman Municipality and to assess consumers' perceptions on the colour of fresh beef as its aesthetic quality.

1.2 Specific objectives

- To assess the prevalence of *Escherichia coli and Salmonella* in fresh beef and contact surfaces at the retail markets in Techiman Municipality.
- To determine post-mortem handling and the hygienic level of beef production in the Techiman Municipality.
- To assess consumers' perception on the post-mortem handling on the appearance (colour) of fresh beef.

CHAPTER TWO

LITERATURE REVIEW

2.1 World livestock production

Livestock production systems occupy about 30 per cent of the planet's terrestrial surface area and are a significant global asset with a value of at least $1.4 trillion (Steinfeld *et al.*, 2006). The livestock sector is increasingly organized in long market chains that employ at least 1.3 billion people globally and directly support the livelihoods of 600 million poor smallholder farmers in the developing world (Thornton *et al.*, 2006)

Keeping livestock is an important risk reduction strategy for vulnerable communities and livestock are important providers of nutrients and traction for growing crops. Livestock products contribute 17% to kilocalorie consumption and 33 % to protein consumption globally, but there are large differences between rich and poor countries (Rosegrant *et al.*, 2009). Livestock production systems have both positive and negative effects on the natural resource base, public health, social equity and economic growth (World Bank, 2009). Currently, livestock is one of the fastest growing agricultural sub sectors in developing countries with 30 per cent Gross Domestic Product (GDP) (International Fund for Agricultural Development (IFAD, 2010). The demand for livestock products have been largely driven by human population growth, income growth and urbanization. The production response in different livestock production systems has been associated

with science and technology as well as increases in animal population (Delgado, 2005).

2.2 Livestock production in Ghana

Livestock production in Ghana's agriculture contributes largely towards the nutritional requirement, providing draught power, manure to maintain soil fertility and income, particularly for farmers in the northern part of the country. The livestock sector contributes in direct products about 7% of the Agricultural GDP (Statistics, Research and Information Directorate (SRID), 2001), excluding manure and draught power that is provided to the crop sector. Ruminant livestock play a major role in the socio-cultural life of the farming communities as a partial determinant of wealth, payment of dowry, and act as a bank and insurance in times of difficulty. Sheep and goats are often slaughtered for various occasions and functions such as births, funeral and marriages (William *et al.*, 1991; Smith, 1990).

The most prominent cattle breed in the country is the West African Shorthorn (WASH). The name of the breed is generally a descriptive term to cover all the variations of small non-humped cattle, generally black and white in colour but sometimes fawn and white. It is an indigenous tough breed of cattle, thick skin with short fine-boned limbs. Zebu influence in the WASH becomes much more marked towards the northern frontier and especially towards the north-east where the tsetse challenge is much less (Hutchinson, 1962). The Zebir accounts for about half the cattle in the country and has developed a degree of tolerance to tsetse- borne trypanosomiasis.

The Sanga, a natural cross between the WASH and the large humped Zebu cattle, follows the WASH in abundance. The Zebu cattle, which are susceptible to trypanosomiasis, are found mainly in tsetse fly free areas (Oppong *et al.*, 2008).

Table 1: Annual domestic meat production (1000 Metric Tons) 2001-2006

Type of Livestock/Year	2001	2002	2003	2004	2005	2006
Cattle	19.1	18.3	18.5	18. 5	18.9	19.1
Sheep	12.8	13.2	13.6	14	14.5	14.9
Goats	12	12.6	13.9	15.3	15.3	15.6
Pigs	9.7	10.4	10.2	10	9.7	10.6
Poultry	14.6	19.4	21.1	23	22.7	27.2

(SRID, 2001)

2.3 Beef production and consumption

The collective name for meat originating from cattle, sheep and pig is "red meat" Food and Agricultural Organization (FAO, 2005). Lawrie (1985) defines meat as "the flesh of animals used for food" and is also often includes not only the musculature but also organs such as liver and kidney, brains and other edible tissue.

FAO (2005a) stated that meat is also regarded as a perishable product. A foodstuff, Cosmetics and Disinfectants Act (FCDA) (1993) under regulation R 2043 meat is described as: "the clean, sound and wholesome skeletal musculature and fatty tissue of any animal species, including game or bird. Food Safety Standards of Australia and New Zealand (FSANZ) (2013) Code defines meat as the whole or part of any buffalo, cattle, deer, pig, poultry, rabbit or hare, slaughtered other than in a wild state. This definition does not include eggs or fetuses. The term 'meat' refers only to meat flesh (skeletal muscle plus any attached muscle connective tissue or fat), but the FSANZ definition also includes offal (i.e. meat other than meat flesh, including brain, heart, kidney, liver, pancreas, spleen, thymus, tongue and tripe), and excludes bone marrow.

2.4 Slaughtering of animal/abattoir operations

An abattoir is a facility, whether stationary or mobile, at or on which animals are slaughtered or intended to be slaughtered. 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 (MSA, 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" (MSA, 2000). An abattoir is the reverse of an assembling factory that is a pre-manufactured item (slaughter animal) is systematically dismantled (slaughter) to the primary components. The slaughtering process

should be described from the point of holding live animals until chilling of the carcasses (Bekker, 1998)

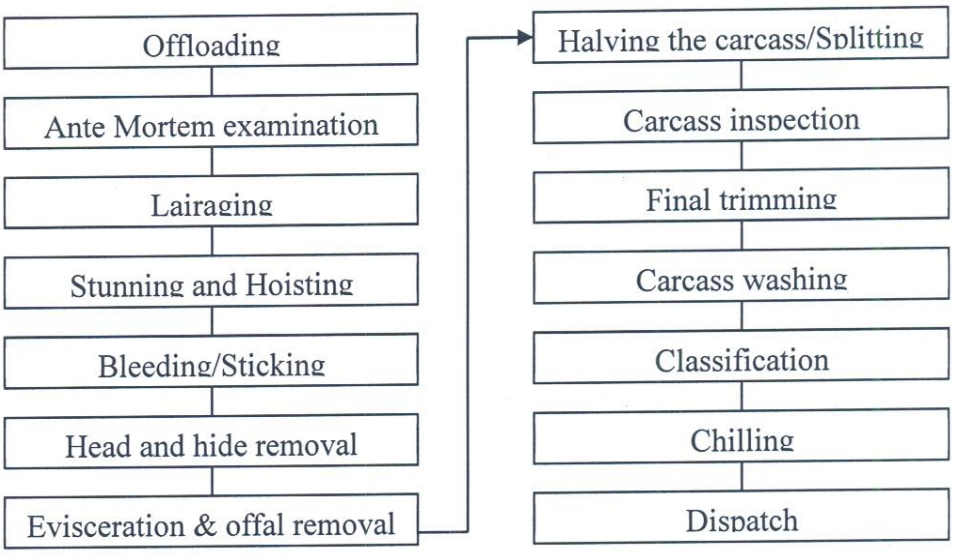


Figure 1: Typical sequence of slaughtering operations for cattle.
(Bekker, 1998)

As long as the processing and handling continues, the greater the danger of contamination of this highly perishable product (meat) (FCDA, 1993). National Health Act (NHA) (2003) defines "contaminate" as "the effect exerted by an external agent on food so that it does not meet a food hygiene standard or requirement determined by any law and does not meet acceptable food hygiene standards or consumer norms or standards and is unfit for human consumption".

2.5 Ante-mortem inspection of live animal for slaughter

On arrival at the abattoir the animals are examined before slaughter by qualified personnel. This is the first opportunity to recognise those animals that may act as

ination, suspected of being infected by a disease or injured animals, therefore eliminating them from slaughter. If the slaughter is not done within 24 hours, the examination must be repeated (Warriss, 2010).

2.6 Contamination during slaughter

The equipment used in killing and dressing (knives, saws, cleavers and direct contact with hides and fleece as well as contact with steels, knife scabbards and the clothing of operatives and hooks), various vessels, receptacles and the personnel may all act as sources of contamination during slaughter (Lawrie, 1991; Kirkpatrick, 2002).

'Dressing procedures currently available cannot be relied upon to prevent or remove all of the bacterial contamination on the carcass surface. Contamination from hides during skinning is considered as the main contributor to the final microbial load of the carcass (Elder *et aL*, 2000). If these procedures are conducted in a correct manner, the degree of contamination can be reduced (Lawrie, 1991; Tricket, 1997).

2.7 Method of bleeding

The religious slaughter methods used by Muslims to produce *halal meat*, is not stunning prior to killing by exsanguinations (Warriss, 2010). All 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 sterilise the knife at 82 °C in between cuts of different animals (MSA, 2000).

2.8 Carcass dressing and evisceration

Traditionally, one or several men would process a single animal at a time, carrying out all the procedures to produce the dressed carcass. Dressing carried out with the animal hanging by its hind legs from overhead rail system from the time it has been exsanguinated with the carcass suspended off the floor was an enormous advance for both hygiene and throughput (Warriss, 2010). After sticking and bleeding the carcasses are dressed. Dressing including the removal of hide, viscera (evisceration), postmortem inspection and cutting the carcass into desired parts such as halving, quartering and primal cuts. Operation system of which production line with each man carrying out a single job has been more efficient and hygienic because, the one de-hiding will not touch inside of the carcass. Also, all equipment and tools will also be restricted to use for one particular purpose and therefore will not be a potential cause of cross-contamination (Warriss, 2010).

2.9 Skinning and de-hiding

Hygienic practices, including frequent hand washing and knife sterilization must be followed with the removal of the hide and contact between the carcass and the

hide must be prevented. Dirty hands, hooks, rollers and protective clothing can contaminate the carcass while removing the hide and must be prevented at all costs (MSA, 2000).

2.10 Evisceration and removal of red and green offal

It is important that the removal of the green offal (ingesta) is carried out properly as the guts are more likely to harbour food-borne pathogens and must be carried out within 45 minutes of bleeding or within 30 minutes if religious slaughtering and within 3 hours if the animal was slaughtered on the farm as a casualty slaughter (Gill *et aL*, 1998).

Bacteria load in the gut is highly significant therefore withholding feed for 24 hours before slaughter is recommended to empty the digestive tract where the heaviest and potentially the most dangerous load of bacteria is located (Pearson and Dutson, 1986; Marriot, 1989). The Food and Agricultural organization stresses the importance of not puncturing the viscera during evisceration, 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 (Lawrie 1991; Thornton and Gracey, 1981; Trickett, 1997). Sterilisation of knives is also very important during this stage. Facilities for the sterilisation of the knives must be provided at this workstation (MSA, 2000).

2.11 Carcass Halving (splitting)

The operator must ensure that the saw is sterilised at 82°C after each carcass and the sterilising cabinet must be in a good functioning condition (MSA, 2000). Halving is done immediately after the animal has been dressed and every effort should be made to saw the carcass into equal sides through the centre of the backbone.

Quartering or ribbing down is the division of a side of a beef between the twelfth and thirteenth ribs into fore-and hindquarters. One rib is usually left on the hindquarter to hold the shape of the loin and make it easier to cut steaks.

2.12 Carcass washing

This step comes in after the final inspection. 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 (FAO, 2005). 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. Excess moisture in the cold room must however be eliminated by means of providing adequate time so that the carcasses can drip-dry to inhibit bacterial growth (FAO, 2005). Bekker (1998) concluded that washing of carcasses does not significantly influence the microbiological load on beef carcasses. Cold water has little effect on bacterial numbers for its effectiveness, than using hot water (80˚C) or including low concentrations of organic acids and chorine (Warriss, 2010).

2.13 Carcass chilling/ cooling

Since lower temperatures reduce or prevent microbial growth, cooling carcass as soon as possible after dressing and keeping meat at low temperatures, can considerably reduce the rate of spoilage and pathogenic bacteria. Fresh meat can normally be stored 5-7 days at refrigerated temperatures (Warriss, 2010). Temperatures of 7 °C and 4 °C are ideal to prevent bacterial growth on carcasses and offal within 24 hours respectively.

The main reason for chilling meat is to reduce or prevent the proliferation of bacteria and certain other microbes such as yeast (Warriss, 2010; Strydom and Buys, 1995) and moulds on meat and to reduce the rate of deteriorative chemical changes e.g. oxidation of fats causing rancidity (Frazier and Westhoff, 2004). Again chilling of meat lengthens its shelf life by slowing down the multiplication of spoilage and pathogenic organisms. The rate of harmful chemical changes, such as rancidity of fat is also reduces by means of chilling (Warriss, 2010). An important point to note is that it is the outside surface of the carcass that is important to cool rapidly because these are where bacterial contamination is likely to be. Meat surface temperatures remain in the growth range for *Escherichia* and *Salmonella* flora for a considerable period. While the initial microbial contamination of meat contains both mesophilic and cold tolerant bacteria, only the second will compete successfully at chill temperatures (Strydom and Buys, 1995).

Two methods of preserving meat through lower temperatures, namely chilling and freezing can be applied. For chilling, meat is stored at a temperature of 0°C to 4 °C and for freezing -18°C. Beef can be stored at -18°C for at least 6-12 months, pork for 6 months and poultry for 3 months (Warriss, 2010). The colder the temperature is, the slower 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 South Africa Meat Industry Companies (SAMIC, 2004).

The important criteria in beef carcass chilling include (1) meat regulations, (2) minimise carcass mass loss, (3) avoid cold shortening of muscle, (4) minimise chilling time to improve throughput (Mallikarjunan and Mitta, 1995) and (5) restriction of microbial growth. Apart from the design and equipment, operational and maintenance requirements, the Meat Safety Act 40 of 2000 of South Africa requires that the carcass shall be placed in a refrigerated room immediately after slaughter

- A minimum post- mortem chilling time of 16 h
- That the temperature of a refrigerated room used for initial chilling of "warm" carcasses, sides or quarters, be maintained at temperatures below

7 °C and the mean air speed over the meat be at a value above 0.75 metres per second. 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, in 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 metres 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%.

2.14 Despatch and transport

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 above-mentioned and will have a direct impact on the shelf-life quality of the meat. Therefore, in terms of section 13(2) and (3) of Regulation R918 promulgated under the South African National Health Act, 2003 (Act 61 of 2003) and 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 (NHA, 2003; MSA, 2000):

"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 and more than 2 °C during local transport (less than 200 km)
- 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 centimetre of the said surface shall upon analysis contain more than 100 viable micro-organisms".

To further prevent contamination, the following transport practices are required in terms of the same legislation:

- "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 as set in these regulations
- Exposed carcasses or meat may not be transported in the same loading space as cartonned 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".

Special care should be taken in order to prevent contamination due to the nakedness of carcasses during the unloading and transportation of meat. This area may be a major source of contamination through handling during loading and unloading and contact with vehicle surfaces (Bekker, 1998). Stringer *et al.* (1969) also claimed that after chilling the amount of contamination increase 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 (Stringer *et al.*, 1969). 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 (FAO, 2005). Before loading proceeds, the meat should be chilled to 0°C. To minimise the temperature rise and to avoid condensation on the meat surface the temperature in these vans can be set and controlled (FAO, 2005).

2.15 Physiological changes at slaughter

Evidence suggests that, when animals are agitated or fatigued, bacteria enter the tissues with ease. Fatigued and stressed animals use up muscle glycogen, which forms lactic acid and changes the pH of the meat tissue (Warriss, 2010).

Therefore, the number of microbes found on the surface of the meat immediately following slaughter would depend on how hygienically the work in the abattoir has been done (Kirkpatrick, 2002). Stringer *et al.* (1969) reported that, immediately after slaughtering, carcasses contain high levels of microbial contamination, and moist carcass areas are highly contaminated. Contamination by contact with unhygienic surfaces, by personnel and airborne organisms, will remain a possibility in all operations during the subsequent handling of the meat (Lawrie, 1991). These will include chilling, freezing, processing, cutting, packaging, transport, sale and domestic handling, although some sources of contamination are obviously removed when the carcasses leave the slaughter floor. It is therefore important to exercise hygiene in slaughterhouses, meat stores, during transport, in wholesale and retail distribution and in the home, to control exogenous contamination (Lawrie, 1991).

2.16 Carcass butchery

Carcasses are sold whole, or as sides, or may be cut into smaller primal or retail joints. The various ways by which a carcass is butchered into joints varies between countries, within countries and depending on the particular use (Warriss, 2010; SAMIC, 2004).

2.17 Wholesale and retail marketing of beef carcasses

According to SAMIC, (2004) live animals are directly sourced by the wholesaler from the farmers on a bid and offer basis. Ownership is therefore taken of the

animals before the animal is slaughtered. The animal is then slaughtered at an abattoir and then carcass is distributed to retailers. In some instances, the public can buy carcasses directly from wholesaler. A beef carcass is halved (split lengthways) and each half of the carcass is quartered into a forequarter and hindquarter. The retail dealer buys beef carcasses in the form of fore and hindquarters from the wholesaler (Warriss, 2010) (plate

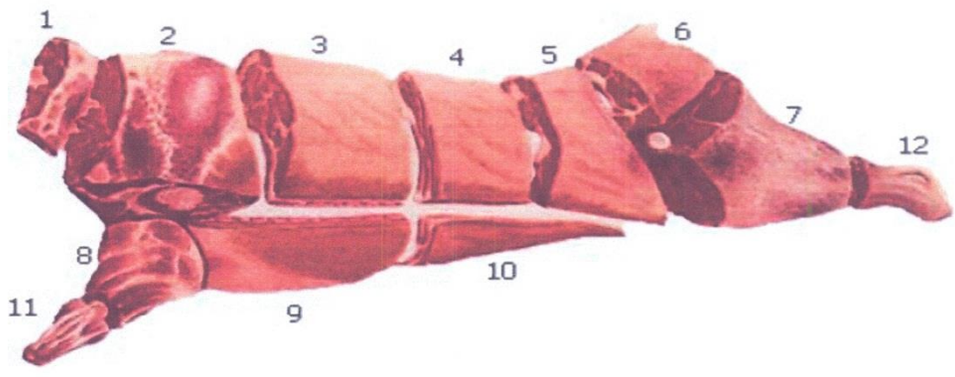


Plate 1: The various primal cuts of the beef carcass

1-Neck; 2-Shoulder, 3-Spinal area 4-Sirloin; 5- Rump; 6-Leg; 7—Aitchbone; 8 - Humeral; 9-Breast; 10-Groin; 11-Front shank; 12-Rear shank

2.18 Contamination during handling and processing

Warriss (2010) indicated 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 beef carcasses can result in product contamination.

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 microorganisms from the cut surfaces. Contamination subsequently occurs by the introduction of microorganisms 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 microorganisms 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 microorganisms whether bacteria, yeasts, moulds, viruses or protozoa (FAO, 2005). Pelczar *et al.* (1986) indicated that fresh meat cut from the chilled carcasses has its surface contaminated with microorganisms 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 (Lawrie, 1991; Frazier and Westhoff, 2004; Trickett, 1997).

According to Marriot (1994), personnel are the largest contamination source and without compliance to sanitary practices, infect food that they touch with spoilage and pathogenic microorganisms. Employees come in contact with these microorganisms 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 microorganisms on a healthy human body (Hobbs and Roberts, 1993; Featherstone, 2003).

Carcass contaminant 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 beef in retail meat display cases (Marriot, 1994; Stivarius *et al.,* 2000).

2.19 Meat quality

There are various major aspects of meat quality: nutritional and wholesomeness qualities which are objective and eating quality as perceived by the consumer to include flavours, juiciness, tenderness and colour which may be highly subjective as well as ethical quality (Warriss, 2010; FAO, 1992; Lawrie, 1991). The wholesomeness quality of meat is increasingly becoming important to consumers due to their health as well as the aesthetic value for money. Campbell and Jopson (2008) described the eating quality of lamb to include traits such as meat and fat colour, pH, tenderness and factors affecting the eating experience such as taste, juiciness and smell. The consumer demands that, meat should be tender, appealing, wholesome, nutritious and affordable, (Warriss, 2010; FAO, 1992; Lawrie, 1991).

The nutritive quality attributes of meat include the nutrient content, nutrient availability and caloric value (Adegoke and Falade, 2005). Meat is high in both protein quality and quantity. According to Bastin (2007), the nine essential amino

acids that the body cannot synthesize are found in meat, thus making it a complete protein. The vitamins and other nutrients that subsist in steaks of pork and chicken include vitamins A, B and D (Callow, 2009). Red meat is a good source of both vitamin B12 and iron and eating moderate amounts of it can reduce anaemia (Reinmuth, 2010). Callow (2009), suggested that consumption of poultry, fish and lean cuts could be the best options to minimize some health hazards that are normally associated with meat.

According to Warriss (2010) Quality can be categorized into two main types as functional quality which refers to desirable attributes in the product. For instance consumers might want red meat to be tender, red coloured and chicken to have good flavour. Conformance quality is producing products that meet the consumer's specification exactly. However both are important because no one wants chicken breast, beef or pork that are exactly the right weight and size but have poor colour, flavour or texture and detriment to their health.

2.20 Microorganisms that infect meat

The microbiological condition of carcass meat is highly dependent on the manner, in which meat animals are reared, slaughtered and processed as well as environmental conditions of the slaughter and processing plant. It is important that only relatively clean animals are presented for slaughtering, since it is extremely difficult to obtain clean meat from dirty animals. Therefore, the cleanliness of livestock depends on husbandry, weather and climate, methods of

transport and holding conditions at the abattoir. Cattle from feedlots may carry more faecal bacteria and less soil organisms (NDA, 2005).

According to Marriot (1994) Food products (including meat) provide an ideal nutrition source for microorganisms and generally have pH values in the range required for proliferation. Meat products are contaminated with soil, air and waterborne microorganisms during slaughtering, processing, distribution and preparation. Extremely high numbers of microorganisms are found in meat animals gastrointestinal tracts, and some of these find their way to the carcass surfaces during slaughter. Apparently some healthy animals may harbour various microorganisms in their organs such as the liver, kidneys, lymph nodes and spleen. These microorganisms and those from contamination through slaughtering can make their way through to the skeletal muscles by way of the circulatory system. Although, some organisms enter the blood and the lymphatic system of live animals there is a natural defensive system ensuring that, there is a state of equilibrium between bacterial attack and bacterial elimination (Bekker, 1998; Frazier and Westhoff, 2004). To a larger extent, the micro-flora of meat will be that of the farm yard which are on the external surfaces of the animal contaminating the meat by direct contact through air, water, soil, manure and the hands and tools of the personnel. The healthy inner flesh of meats has been reported to contain few or no micro-organisms, although *Salmonella, Staphylococci, Streptococci* and *Clostridia* have been isolated from lymph nodes, bone marrow, and even flesh (Bekker, 1978; Frazier and Westhoff, 2004). Psychrotrophic strains of *Achromobacter, Micrococcus, Flavobacterium and*

Pseudomonas, were isolated from the carcasses after dressing. Nevertheless, the hide remains an important source of microorganisms for contamination of the carcass (Jay, 1992). Psychrotrophic bacteria, the group that includes potential spoilage bacteria for chilled meat, are common in soil, water and vegetation (Newton *et al.,* 1978).

Gill and Newton (1980) reported that, usually the most predominating under aerobic conditions are, *Moraxella spp., Acinetobacter spp., Microbacterium thermosphactum* and members of the *Enterobacteriaceae.* On the other hand, members of the bacterial genera *Aeromonas, Alcaligenes, Arthrobacter, Brochothrix, Brucella, Campylobacter, Corynebacterium, Enterobacter, Escherichia, Flavobacterium, Hafnia, Listeria, Microbacterium, Pediococcus, Salmonella, Staphylococcus and Yersinia* can be recovered from red meat and their products to a lesser extent (Gill and Newton, 1980).

The pathogenic mesophiles such as *Salmonella,* which have been observed to grow on meat at 25°C, are of a particular concern (Nolte and Naude, 1981; Gill and Newton, 1980).

2.21 Bacteriological quality/contaminations and specific genus of mycobacterium

Microorganisms are considered the source of all life forms, enormously abundant, more phylogenetically diverse than plants and animals, and grow in virtually every place on earth. Furthermore, they carry out transformations of

matter essential for life, affect climate, participate in countless symbiotic relationships with plants, animals and other microbes, cause diseases, and influence the behaviour of animals and plants (Jay *et al.*, 2005). Microorganisms can be used as source of food or food supplements to other foods as a result they are described as unicellular protein (Prescott *et al.*, 2002). However microorganisms and other infectious agent had caused and continue causing loss of several lives (Nester *et al.*, 2001).

Microorganisms that affect meat quality are categorized into moulds, yeast and bacteria (Jay *et al.*, 2005).

2.22 Moulds

Most moulds are visible and multicellular. They exist in different forms on their substrates like the white fluffy patches on tomato paste, blackened areas along the rubber lining on refrigerator doors etc. These are filamentous fungi that grow in the form of a tangle mass that spread rapidly and may cover several inches of areas in two to three days. The total mass or large portion is termed as mycelium and this is composed of branches or filament referred to as hyphae (fine threadlike strands of moulds). Beneficial moulds are used in the production of cheese e.g. *asocospores, zygospores* or *conidia* and antibiotics (penicillin) while the non-beneficial moulds cause food spoilage, skin infections e.g. ringworm and sometimes liver cancer through aflatoxin production (Jay *et al.*, 2005)

2.23 Yeast

Yeast is a non-taxanomic category of fungi defined in terms of morphological and physiological criteria. The typical yeast (e.g. *Saccharomyces cerevisae) is* a unicellular saprotroph which can metaboliszed carbohydrates by fermentation. Yeasts are generally larger than bacteria and reproduce by budding (Singleton and Sainsbury, 2006). Budding is an asexual reproduction method whereby an outgrowth develops on a parent cell or mother well which eventually cuts off to form new daughter cell However some yeast e.g. *schizosaccharomyce spp*, divides by fission and some can form a pseudomycelium or a true mycelium e.g. *Hansnula canadensis, Lipomyces spp, Sporobolomyces spp* are non-fermentive, and some are pathogenic e.g. *Candida albicans*. Beverages and bread manufacturers use yeast to breakdown sugars to CO2 and alcohol (Singleton and Sainsbury, 2006).

2.24 Bacteria

A taxon comprising one of the two basically distinct groups of prokaryotic microorganisms. Bacteria are a diverse group of unicellular organisms. Most are free-living, occurring in soil, on plants, in various aquatic habitats and even in Antarctic snow. Bacteria are also found as symbionts in plants, animals and certain microorganisms (Singleton and Sainsbury, 2006). According to Warriss (2010) bacteria are classed as gram-negative or gram-positive, based on their reaction with various dyes. Gram-positive bacteria retain the stain, crystal violet, while gram-negative do not. The major difference between the two sorts of

bacteria is that, Gram-negative bacteria have thin walls of peptidoglycan with a second protective outer surface membrane while gram-positive bacteria have walls thickened with polysaccharides and proteins Public Health Veterinarian (PHV, 2011).

2. 25 Selected organisms for this study

Previous studies in Tamale (Sulley *et al.,* 2006), Bolgatanga (Adzitey *et al.,* 2011) and Accra (Soyiri *et aL,* 2008) indicated that pathogenic bacteria are transferred to carcasses through handlers, equipment and poor sanitary practices. The importance of this determination is more urgent given the implementation of the "Pathogen Reduction" performance standards by the Food Safety and Inspection Service (FSIS, 1994). These standards require random sampling of meat and poultry carcasses for generic *E. coli* and *Salmonellae.* Generic *E. coli* has been characterized as a target organism as its presence indicates faecal contamination, its virulence produced in human and the few number of infective doses and therefore samples should be tested by an establishment at a frequency according to its production volume. The sampling of meat and related facilities for *Salmonellae* is because "it is;

1) the most common cause of bacterial food-borne illness;

2) FSIS baseline data show that *Salmonella* colonizes a variety of mammals and birds, and occurs at frequencies which permit changes to be detected and monitored;

3) current methodologies can recover *Salmonella* from a variety of meat and poultry products

4) intervention strategies aimed at reducing *Enterobacteriaceae* or faecal contamination and other sources of *Salmonella* on raw product should be effective against other pathogens" (Federal Register, 1996).

2.26 *Salmonella spp.*

Salmonella spp. are bacteria that cause salmonellosis, a common form of foodborne illness in humans. Outcomes from exposure to *Salmonella* spp. can range from mild symptoms to severe disease and can be fatal. *Salmonella* spp. are carried by a range of domestic and wild animals and birds and have been widely isolated from the environment (FSANZ, 2013). *Salmonella* spp. are Gram-negative, non-spore forming rod-shaped bacteria and are members of the family *Enterobacteriaceae* (Jay *et al.,* 2003). The genus *Salmonella* is divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori*. Over 99% of human *Salmonella* spp. infections are caused by *S. enterica* subsp. *enterica* (Bell and Kyriakides, 2002; Crum-Cianflone, 2008).

Strains of *Salmonella* can be characterised serologically (into serovars) based on the presence and/or absence of 0 (somatic) and H (flagella) antigens. Phage typing is used to subtype *Salmonella* serovars. The phage type is determined by the sensitivity of the bacterial cells to the lyric activity of selected bacteriophages (Bell and Kyriakides, 2002; Jay *et al.,* 2003).

Salmonella typhi and *Salmonella paratyphi* are specifically associated with infections in humans, leading to severe disease called enteric fever. *S. typhi* and *S.*

paratyphi produce clinical syndromes referred to as typhoid and paratyphoid fever, respectively. Enteric fever is rare in developed countries, with the majority of cases associated with overseas travel (Darby and Sheorey, 2008).

Salmonella spp. have relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates. The temperature range for growth of *Salmonella spp.* is 5.2-46.2°C, with the optimal temperature being 3543°C (International Commission on Microbiological Specification for Food (ICMSF), 1996). Although freezing can be detrimental to *Salmonella spp*. survival, it does not guarantee destruction of the organism. There is an initial rapid decrease in the number of viable organisms at temperatures close to the freezing point as a result of the freezing damage. However, at lower temperatures *Salmonella spp*. have the ability to survive long term frozen storage (Jay *et al.*, 2003).

Salmonella spp. will grow in a broad pH range of 3.8-9.5, with an optimum pH range for growth of 7-7.5 (ICMSF, 1996). The minimum pH at which *Salmonella spp*. can grow is dependent on temperature, presence of salt and nitrite and the type of acid present. Volatile fatty acids are more bactericidal than organic acids such as lactic, citric and acetic acid. Outside of the pH range for growth, cells may become inactivated, although this is not immediate and cells have been shown to survive for long periods in acidic products (Bell and Kyriakides, 2002; Jay *et al.*, 2003). Water activity (a_w) has a significant effect on the growth of *Salmonella spp.*, with the optimum a_w being 0.99 and the lower limit for growth being 0.93. *Salmonella spp*. can survive for months or even years in foods with a low a_w (such as black pepper, chocolate, peanut butter and gelatine) (ICMSF, 1996).

Salmonella spp. are similar to other Gram negative bacteria in regard to susceptibility to preservatives commonly used in foods. Growth of *Salmonella spp.* can be inhibited by benzoic acid, sorbic acid or propionic acid. The inhibition of *Salmonella spp.* is enhanced by the use of a combination of several preservative factors, such as the use of a preservative in conjunction with reduction in pH and temperature (ICMSF, 1996; Banerjee and Sarkar, 2004). *Salmonella spp.* are classed as facultative anaerobic organisms as they do not require oxygen for growth (Jay *et al.*, 2003).

Outcomes of exposure to *Salmonella spp.* can range from having no effect, to colonisation of the gastrointestinal tract without symptoms of illness (asymptomatic infection), or colonisation with the typical symptoms of acute gastroenteritis. Gastroenteritis symptoms are generally mild and may include abdominal cramps, nausea, diarrhea, mild fever, vomiting, dehydration, headache and/or prostration. The incubation period is 8-72 hours (usually 24-48 hours) and symptoms last for 2-7 days (Darby and Sheorey, 2008). Severe disease such as septicaemia sometimes develops, predominantly in immunocompromised individuals. This occurs when *Salmonella* spp. enter the bloodstream, leading to symptoms such as high fever, lethargy, abdominal and chest pain, chills and anorexia; and can be fatal. A small number of individuals develop a chronic condition or sequelae such as arthritis, appendicitis, meningitis or pneumonia as a consequence of infection (Hohmann, 2001; Food and Drug Administration (FDA), 2012).

People of all ages are susceptible to *Salmonella* spp. infection. However, the elderly, infants and immunocompromised individuals are at a greater risk of

infection and generally have more severe symptoms (Jay *et al.*, 2003; FDA, 2012). *Salmonella* spp. are transmitted by the faecal-oral route by either consumption of contaminated food or water, person-to-person contact, or from direct contact with infected animals (Jay *et al.*, 2003).

Salmonellosis is one of the most commonly reported enteric illnesses worldwide, being the second most frequently reported cause of enteric illness in Australia (National Notifiable Disease Surveillance System (NNDSS, 2013).

Outbreaks attributed to *Salmonella spp*. have predominantly been associated with animal products such as eggs, poultry, raw meat, milk and dairy products, but also include fresh produce, salad dressing, fruit juice, peanut butter and chocolate (Jay *et al.*, 2003; Montville and Matthews, 2005).

The primary reservoir of *Salmonella spp*. is the intestinal tract of warm and cold-blooded vertebrates, with many animals showing no sign of illness. Unlike diseased animals which can be removed from production and/or treated, these asymptomatic (carrier) animals can shed large numbers of *Salmonella spp.* in their faeces and are therefore an important source of contamination. Faecal shedding of *Salmonella* spp. leads to contamination of the surrounding environment including soil, crops, plants, rivers and lakes. A wide range of foods have been implicated in foodborne salmonellosis, particularly those of animal origin and foods that have been subject to sewage pollution (ICMSF, 1996; Jay *et al.*, 2003).

At the time of slaughter, *Salmonella* infected animals may have high numbers of organisms in their intestines as well as on the outside of the animal (faecal contamination of hides, fleece, skin or feathers) (Bryan and Doyle 1995; Jay *et*

al., 2003). In Australia, *Salmonella spp.* have been isolated from 3% of chilled cattle carcass (Fegan *et al.,* 2005). The distribution of *Salmonella spp*. on contaminated meat carcasses is not uniform. For example, Stopforth *et al.* (2006) found that the prevalence of *Salmonella* spp. on fresh beef ranged from 0.8% (rib eye roll) to 9.6% (strip loins,) depending on the cut of meat. Cross-contamination during processing may also lead to increased prevalence of *Salmonella* in finished products (Bryan and Doyle, 1995)

2.27 *Escherichia coli* 0157:H7

Escherichia coli are normal inhabitant of the intestine of all animals, including humans; serves a useful function in the body by suppressing the growth of harmful bacteria species and by synthesizing appreciable amounts of vitamins. *E. coli* has been used since 1890 as a non-pathogenic indicator of enteric pathogens, such as *Salmonella*. However, as knowledge of enteric diseases increased, investigators began isolating strains of *E. coli* that had acquired virulent characteristics causing pathogenicity to humans or animals. A minority of *E. coli* serotypes are capable of causing human illness (colibacillosis) by different mechanisms. *E. coli* 0157:H7 was first recognized as a human pathogen following two hemorrhagic colitis outbreaks in 1982 (Riley *et al.,* 1983). Undercooked hamburgers from the same fast food restaurant chain were identified as the vehicle, and *E. coli* 0157:H7 was isolated from patients and a frozen ground beef patty. Shortly after *E. coli* 0157:H7 was determined to be a human pathogen; Karnali *et al.* (1983) observed that stool samples from children with

hemolytic uremic syndrome (HUS) contained a substance that was toxic to Vero (African green monkey kidney) tissue culture cells. This verocytotoxin was produced by *E. coli* isolates, with 0157117 the prominent serotype causing infection.

Based on disease syndromes and other characteristics, the following classes of *diarrheagenic E. coli* have been recognised: *enteroinvasive* (EIEC), *enteropathogenic* (EPEC), *enterotoxigenic* (ETEC), *enterohemorrhagic* (EHEC), and diffusely adherent (DAEC). EHEC is the class that is of concern to industry, FSIS, and public health; the more significant serotype is *E. coli* 0157117 (IFT, 1997).

Like all bacteria, the survival and growth of *E. coli* 0157:H7 in foods are dependent on the interaction of various intrinsic and extrinsic factors such as temperature, pH, and water activity. EHEC strains respond to temperature in the same manner as non-EHEC strains, with the exception of isolates of serotype 0157:H7. *Escherichia coli* are differentiated from other *Enterobacteriaceae* (family of Gram-negative, catalase-positive, oxidase-negative, facultatively anaerobic rods) on the basis of their ability to grow and produce gas in EC *(E. coil)* broth at 44.5°C. Many 0157:H7 isolates, however, do not grow well, if at all, above 44°C (Doyle and Schoeni, 1984). Palumbo *et al.* (1995) found that the upper temperature for *E. coli* 0157:H7 growth was culture medium— dependent; all strains grew in brain heart infusion (BHI) broth at 45°C, but six of sixteen strains did not grow in EC broth.

The minimum growth temperature for *E. coli* 0157:H7 under otherwise optimal conditions is approximately 8-10°C (Buchanan and Bagi, 1994; Rajkowski and Manner, 1995). Growth rates are similar at pH values between 5.5 and 7.5, but

decline rapidly at lower pH values (Buchanan and Klawitter, 1992). The minimum pH for *E. coli* growth is 4.0-4.5 (Buchanan and Bagi, 1994). This is dependent on the interaction of pH with other growth parameters; for example, additional stresses raise the minimum pH for growth. The type of acid (e.g., organic vs inorganic), and acid concentration influence the effect of pH on *E. coli* growth. Abdul-Raouf *et al.* (1993) reported that, in beef slurries, the relative inhibitory activity of organic acids on *E. coli* 0157: H7 was acetic > lactic > citric. When the pH falls below the minimum for growth, *E. coli* 0157: H7 population decline over time. The pathogen has been shown experimentally to survive for several weeks to months in a variety of acidic foods, including mayonnaise (Zhao and Doyle, 1994), sausages (Clavero and Beuchat, 1996) apple cider (Zhao *et al.*, 1993), and Cheddar cheese (Reitsma and Henning, 1996). Survival in these foods is extended greatly when stored at refrigeration temperature. For example, the pathogen survived in apple cider for only 2-3 days at 25°C, compared to 10-31 days at 8°C (Zhao *et al.*, 1993). EHEC strains can have a high degree of acid tolerance, surviving virtually unchanged during 2- to 7-hr exposures at pH 2.5 and 37°C (Benjamin and Datta, 1995; Buchanan and Edelson, 1996).

Acid-sensitive EHEC strains, however, have also been identified. Lin *et al.* (1996) examined three mechanisms of acid resistance, i.e., oxidative, arginine-dependent, and glutamate-dependent, and found that all three contribute to the microorganism's overall acid tolerance. Induction of acid tolerance in E. coli can enhance its survival in acidic foods (Cheville *et al.*, 1996; Leyer *et al.*, 1995). An acid tolerant state can persist for extended periods (28 days) if the cells are stored

at refrigeration temperature. The induction of acid tolerance can also enhance the organism's ability to survive other stresses. Recent report indicated that induction of acid tolerance also increases the microorganism's resistance to heating, radiation, and antimicrobials (Rowbury, 1995). *E. coli* also possesses an inducible alkali tolerance response (Rowbury *et al.*, 1996).

Studies on the effect of water activity on the survival and growth of *E. coli* 0157:H7 focused primarily on the effect of sodium chloride, though, presumably, *E. coli* 0157:H7 behaves similarly to other *E. coll.* Buchanan and Bagi (1994) developed a mathematical model for the effects and interactions of NaC1 concentration (0.5-5.0%) with temperature, pH, and NaNO2 on the growth kinetics of *E. coli* 0157:H7. They compared the effects of mannitol, sorbitol, and sucrose as humectants and concluded that while between humectant differences occur at limiting a_v, values, differences among humectants were minimal at a_w 0.98 (Buchanan and Bagi, 1997). *E. coli* 0157:H7 can survive for many weeks when desiccated, particularly at refrigeration temperature (Bagi and Buchanan, 1993). *E. coli* 0157:H7 does not appear to have any increased resistance to antimicrobial food additives.

This pathogen produces several virulence factors that cause severe damage to the lining of the intestine, intravascular destruction of red blood cells

(microangiopathic hemolytic anemia), depressed platelet counts

(thrombocytopenia), lack of urine formation (oligo-anuria), swelling (edema), acute renal failure and neurological problems (Boyce *et al.*, 1995). Hemolytic

Uremic Syndrome (HUS) occurs most often in children under the age of 10, especially 6 months to 5 years. Approximately half of HUS patients require

dialysis, and the mortality rate is 3-5%. Other HUS-associated complications may include seizures, coma, stroke, colonic perforation, pancreatitis, and hypertension. Approximately 15% of cases lead to early development of chronic kidney failure. Insulin-dependent diabetes may also persist in HUS patients. A small number of HUS cases may recur (Siegler *et al.*, 1993).

Since 1982, *E. coli* 0157:H7 has been implicated in outbreaks worldwide and is the primary cause of hemorrhagic colitis (HC) and hemolytic uremic syndrome

(HUS) in the United States, Canada, Great Britain, and regions in Europe. The pathogen is likely responsible for 85-95% of HUS cases (Griffm, and Tauxe, 1991). This has led to classification of EHEC largely on the basis of characteristics of serotype 0157:H7.

This microorganism causes three distinctive clinical manifestation including hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic

thrombocytopenic purpura (UP). All people are believed to be susceptible to hemorrhagic colitis, but young children and the elderly appear to progress to more serious symptoms more frequently (HUS and TTP, respectively).

Ground beef has been the food most often associated with outbreaks in the United States (Griffin and Tauxe, 1991). A significant portion of HC infections are sporadic, i.e., not associated with outbreaks. Ground beef also has been implicated as a risk factor in those sporadic infections (Le Saux *et al.*, 1993).

Dry-cured salami was associated with an *E. coli* 0157:H7 outbreak in the western United States, demonstrating that low level of this organism can survive in acidic fermented meats and cause illness (Tilden *et al.*, 1996). EHEC isolates of serotypes 0111: H— and 0157: H— were isolated from both patients and product

(Paton *et al.*, 1996). *E. coli* isolates capable of producing one or more Shiga toxins can be isolated readily from meat, poultry, and sea foods (Samadpour *et al.*, 1994); however, most do not possess the other virulence determinants associated with fully pathogenic EHEC.

Escherichia coli 0157:H7 is a bacterial pathogen that has a reservoir mainly in cattle; other reservoirs have been identified including pigs, sheep, flies, deer and other wild animals. It has been shown that feedlot steers and heifers appear to carry the organism at higher levels than once thought, even higher than dairy cattle and calves Institute of Food Technology (IFT, 1997). Undercooked or raw hamburger (ground beef) has been implicated in many of the documented outbreaks. Because of its public health significance, the vast scientific evidence showing the high incidence in cattle, the severity of the illness, and outbreaks due to this pathogen, FSIS (1994) declared *E. coli* 0157:H7 to be an adulterant in ground beef products. Additionally, *E. coli* 0157:H7 outbreaks have also implicated alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, spinach, lettuce, game meat, cheese curds, among others.

Other vehicles of infection with *E. coli* 0157:H7 include person-to person transmission (Griffm and Tauxe, 1991), water (recreational, well, and municipal water systems) (Doyle *et al.*, 1997), animal contact (farms and petting zoos), and diagnostic laboratory related.

2.28 Refrigeration (chilling and freezing)

Temperature control is mainly achieved through chilling and freezing. For chilling, meat is stored at a temperature of 0°C to 4°C and for freezing at -18°C (Warriss, 2010). Temperature preservation inhibits the microbiological and other effects that lead to spoilage (Warriss, 2010). According to Warriss (2010) beef and lamb stored at -18°c last for 6-12 months while pork and poultry can be stored for 6 and 3 months respectively. The quality and safety of the final product are determined by the initial quality of the muscle, hygiene standards during dressing and processing, temperatures throughout processing operations and finally the duration of and environmental conditions during the chilling and storage phase. Therefore, the cold chain should be strictly maintained throughout the meat production chain Food Advisory Consumer Service (FACS, 2004). FACS (2004) describes the "cold chain" as the whole distribution line of chilled or frozen food from raw material to final consumption.

Chilling at core temperatures (7°C and -18°C) will not prevent the activity of spoilage organisms, which can grow at 7°C or lower. However, temperatures below 2 °C will delay the onset of slime formation, slow the enzyme action and the growth and development of bacteria, hence a slower putrefaction rate. The nutritional value, appearance and taste of meat are retained by refrigeration and freezing. This is only possible if the meat is hygienically packed and sealed and refrigerated at a constant temperature of 0°C to 4°C or frozen at -18°C (SAMIC, 2004). Gill and Newton (1980) indicated that most meat will be exposed to room

temperatures at some time and may be held for extended periods without refrigeration if such facilities are not available.

According to Frazier and Westhoff (2004), chilled storage is at temperatures not far above freezing and usually involves cooling by ice or by mechanical refrigeration. It may be used as the main preservative method for foods or for temporary preservation until some other preservative process is applied. Most perishable foods, including eggs, dairy products, meats, seafood, vegetables and fruits may be held in chilling storage for a limited time with little change from their original state. Modern packinghouse methods involve chilling meat promptly and rapidly to temperatures near freezing and chilling storage at only slightly above the freezing point. Mallikarjunan and Mittal (1995) define chilling time, as the time required to lower the temperature at the geometric centre of the round below 5°C. According to Shapton and Shapton (1991) the time the carcass is held in the chiller, may have a more significant effect on the microbial population than the chill temperature. Mallikarjunan and Mittal (1995) gave important criteria in beef carcass chilling as:

- "Meeting regulations
- Minimising carcass mass loss. To minimise the mass loss, the carcass surface temperature should be lowered as fast as possible
- Avoiding cold-shortening of muscles. Unless carcasses are electrically stimulated, cold shortening can be avoided by holding the muscle temperature above 10°C up to 10 hours post mortem.

- Minimising chilling time to improve throughput. However, rapid chilling has caused cold shortening in pre-rigor muscles and reduced the surface temperature of the carcass below initial freezing point".

Chilling is used for short-term storage, while freezing is used for long term preservation of meat. Meat must be kept as close to 0°C during chilling as possible without actually freezing it. The ideal chilling room will create a firm, dry carcass surface, where the risk of contamination during handling and transport will be much less with a minimum mass loss of carcasses. It will also inhibit the growth of surface bacteria (Warriss, 2010). During chilling both air temperature and humidity must be carefully controlled. The humidity must be maintained at about 90-95% (Warriss, 2010). If the humidity is too high, the carcass will not dry, and if it is too low excessive dehydration and darkening of the meat will take place (RMAA, 2004).

According to Nortje and Naude (1981) adequate chilling adhering to the mentioned rules will:

- control the proliferation of bacteria and certain other microbes such as yeast and moulds
- prolong the shelf life by slowing down the multiplication of organisms, which cause meat spoilage.
- slow down the multiplication of organisms which cause food poisoning
- reduce the rate of chemical changes such as rancidity of fats
- improve handling qualities.

Deep muscle tissue from healthy animals slaughtered under normal hygienic conditions is usually sterile, so that spoilage at both chill and room temperatures will generally result from growth of bacteria on the meat surface only (Gill and Newton 1980). Shapton and Shapton (1991) stated that fast chilling at low temperatures with high air speed and low humidity, may reduce bacterial numbers. The more prompt and rapid this cooling, the less opportunity there will be for growth of mesophilic micro-organisms with storage temperatures between - 1.4 to 2.2°C (Frazier and Westhoff, 1988). Under less severe conditions, growth of psychrotropic organisms can alter the proportion of psychrotrophs to mesophiles. Carcasses cooled at ambient temperature of 15-20°C must be expected to show growth of mesophiles, including pathogens (Shapton and Shapton, 1991).

When the number of bacteria on the surface of chilled carcasses, reaches a count of 6 x 10^1 to 10^8 per cm^2 , the meat develops an off-odour, discolours and finally the surface becomes sticky or slimy (Nortje and Naude, 1981). At this phase, the slime limit is closely correlated to the bacterial count at the beginning of storage. The initial microbial count greatly influences meat shelf life (Nortjd and Naude, 1981).

Chilling and freezing can have potential effects on meat colour, WHC and other aspects of quality. Thus chilling influences the rate of penetration of oxygen from surface of lean meat, and the rate of enzymic reducing activity in the muscle and combats metmyoglobin formation. Meat colour is therefore brighter if is held at lower temperature because the thickness of the surface oxymyoglobin layer is

correspondingly greater (Warriss, 2010). Marriot (1994) indicated that although chilling is one of the most important methods for reducing the effects of contamination during commercial food processing and distribution, correct techniques for cold storage are frequently not followed, and food contamination may occur. Growth rate of microorganisms may therefore increase tremendously at temperatures slightly above minimal temperature for growth.

2.3.0 Aesthetic quality of fresh beef

2.3.1 Meat processing and consumer perception

Meat processing involves slaughtering animals and butchering the meat for retailing or for processing (canning, curing, freezing,) or manufacturing of meat products (Toxtown, 2010). It includes preparing by-products such as lard, gelatin or tallow. Processed meat products are defined as those products in which properties of fresh meat have been modified using one or more procedures, such as mincing or chopping, addition of seasonings, alterations of colour or heat treatment (Aberle *et al.*, 2001). These modifications contribute to preservation, convenience, appearance, palatability, variety and safety giving the consumer a wide choice of meat products. Initially meat was processed to safeguard it, but by virtue of the various procedures, processing leads to so many changes in texture and flavour which adds variety to the diet (FAO, 1992). According to Aberle *et al.* (2001) the present day manufacture of processed meat products is driven largely by consumer demand for safety, appearance, convenience, unique flavour,

distinctive product forms original packaging and aesthetic value. This gives the consumer the prospect in determining preference and hence value for money (Aberle *et al.*, 2001).

Over the last twenty years, the positive image of the nutritional value of red meat has been overshadowed by diverging developments in the market and the meat sector (Scollan *et al.*, 2006). Consumers have been increasingly concerned about food-risks and personal health, particularly hygiene and quality and require detectable indications such as health certificates at the market place or veterinary stamps at the butcher stage (Zaibet *et al.*, 2007). Consumers, consider food safety risk to include spoilage and residues such as microbes (Brewer *et al.*, 1994; Brewer and Prestat, 2002). This has resulted in an increase in demand for safe and healthy foods. Essential stimulus such as colour, marbling fat etc are known to carry more weight when consumers form meat quality expectations than do extrinsic cues (packaging, number of servings etc) and must therefore be entirely satisfied with the sensory properties of a product before other quality dimensions become pertinent (Chambers and Bowers, 1993). Wolfe (1998) suggested that, in order to address the expectations of consumers, the health benefits associated with eating low fat products as well as the idea or concepts of freshness and taste need to be incorporated into any new promotional campaign to meet the new trend in consumer preference.

2.4.0 Appearance (colour) of fresh beef

2.4.1 Definition of Colour and its perception

Colour is the characteristic of a visible radiant power by which an observer may distinguish differences between two structure-free fields of view of the same size and shape, such as may be caused by differences in the spectral composition of the radiant power (colour stimulus) entering the eye (Wyszecki and Stiles, 1982). The energy that produces colour is contained in light. 'Pigments' are molecules that absorb some of the wavelengths from the light that illuminates an object. Both the pigments in the product and the light directed at the product determine how colour of the object appears.

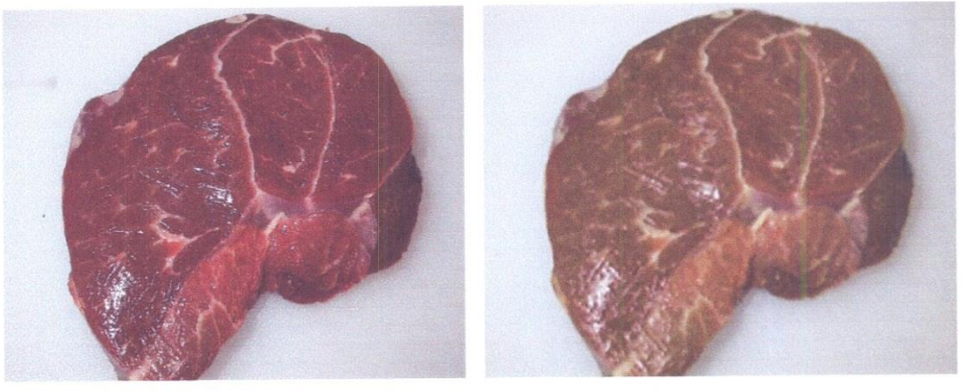
Colour is used to judge the 'value' or quality of a product, when evaluating and choosing a product based on expectations and past experiences. Preferences made based on visual assessment, which requires no physical contact, cause very little menace; therefore, when a product does not meet 'colour expectations' it is a simple decision to consider a product `unacceptable'; (Mancini and Hunt, 2005)

Colour is the single most important factor influencing meat purchasing decisions of consumers more than any other quality factor because consumers use colour as an indicator of freshness and wholesomeness. In a recent consumer survey, 41% of customers rejected meat that appeared brown, even when the use by date had not been exceeded. As a consequence retailers often discount meat to prevent the display period extending beyond 2 days. Nearly 15% of retail beef is discounted in price due to surface discolouration, which corresponds to annual revenue losses of $lbillion (Mancini and Hunt, 2005; Adegoke and Falade, 2005; Smith *et al.*, 2000; Robin *et al.*, 2008).

An economic improvement associated with product that achieves its colour life potential depends on knowledge of pre- and post-mortem myoglobin chemistry. Carpenter *et al.* (2001); Hood and Riodan (1973) made an observation of strong association between colour preference and purchasing intent with consumers discriminating against beef that is not red and bright in colour (purple or brown beef). Therefore, visual determinations are the gold standard for assessing treatment effects and estimating consumer perception (Knopf, 1980).

The stability of meat colour is affected by a number of on-farm, processing and retail display practices. Changing some of these practices along the supply chain can therefore improve colour stability, and hence shelf-life, with potentially significant cost savings at the retail level (Robin *et al.*, 2008).

When meat is sliced, it absorbs oxygen from the atmosphere at the cut surface. Within one hour this process is complete and the surface of the meat is usually bright red; at this stage it is known as the "bloom colour". However as time progresses, the surface of sliced meat will change from red to brown due to oxidation of the pigment from oxymyoglobin to metmyoglobin as shown in plate 2 a and b below. B is unattractive to consumers. That is a lamb leg chop showing the change in colour with time from red to brown as the pigment in the meat surface changes from oxymyoglobin to metmyoglobin.



A B

Plate 2: Mutton pigment change from red (oxymyoglobin) to brown (metmyoglobin) (Robin *et al*., 2008)

2.4.2 Meat pigments

Myoglobin and hemoglobin are the pigments giving meat the red colour (Govindarajan, 1973; Giddings, 1977). Myoglobin is distributed uniformly throughout the muscle, and its role is to store and to facilitate the diffusion of oxygen from the capillaries to the intracellular structures, where the oxygen is used for oxidative processes (Stryer, 1981; Ledward, 1992). Hemoglobin, which is contained in the red blood cells, serves as the oxygen carrier in blood (Stryer, 1981). The myoglobin content in muscle depends on species, breed, sex, age, type of muscle, level of training (Ledward, 1992) and altitude (Gimenez *et al.*, 1977). Myoglobin is present in the sarcoplasmic fraction of the muscle (Govindarajan, 1973) and evenly distributed across the muscle fibres (Swatland, 1984).

Myoglobin is a compact globular protein consisting of globin (apo protein) and an iron containing heme group, Fe-protoporphyrin, which is the chromophore of myoglobin (Stryer, 1981). The characteristic colours of myoglobin upon interaction with light depend on the ligand bound to the chromophore. Hemoglobin is composed of four globin molecules with four heme groups. The iron atom binds to the four nitrogens in the centre of the protoporphyrin ring and can form two additional bonds, one on either side of the heme plane, the fifth and sixth coordinating positions. The iron atom can be in several redox states with the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) redox states being most important in relation to fresh meat colour. The fifth coordinating position is bound to histidine on the globin, and the sixth is free for binding to different small ligands such as 02, H2O, OH-, NO and CO (Hamm, 1975; Stryer, 1981). The colour of myoglobin is determined by the redox state and by the type of ligand bound (Govindarajan, 1973; Hamm, 1975). Oxygen can only be bound to myoglobin in the ferrous redox state, whereas H2O is bound in the ferric redox state at physiological pH and below (Govindarajan, 1973). The different myoglobin species in fresh meat are listed in table 2 below.

Table 2: Chemicals species of myoglobin

Myoglobin species	Oxidation state	Ligand	Colour
Deoxymyoglobin (Mb)	$Fe2^{+}$	None	Purple
Oxymyoglobin (Mb02)	$Fe2^{+}$	02	Bright cherry red
Metmyoglobin (MetMb)	$Fe3^{+}$	H2O [pH<8]	Brown

The heme group is a flat molecule bound to myoglobin in a hydrophobic crevice, protecting the iron from oxidation (Stryer, 1981). The iron is more prone to oxidation if the heme group is detached from the globin (Govindarajan, 1973). The colour of fresh meat is determined by the concentration and chemical nature of the hemoproteins present (Govindarajan, 1973) and the muscle structure (Offer *et al.*, 1989), which is influenced by temperature/pH history of the muscle post-slaughter (Ledward, 1992). In most meats, myoglobin is the main heme pigment although hemoglobin may be present in significant concentrations and to some extent mitochondrial cytochrome *c* (Hamm, 1975; Ledward, 1992).

2.4.3 Myoglobin oxygenation, oxidation and reduction

The colour cycle of fresh meat is reversible and dynamic with constant interconversion of mainly the three species: deoxymyoglobin (Mb), oxymyoglobin (Mb02) and metmyoglobin (MetMb) (Govindarajan, 1973). The surface colour changes from purple to bright red, due to oxygenation of Mb to Mb02, when fresh meat is exposed to oxygen, a reaction known as blooming occurs (Govindarajan, 1973; Giddings, 1977). The reaction is reversible with oxygen partial pressure determining the partition between the two species

(Giddings, 1974). The oxygen binding of Mb only shows a small effect with changes in pH (Govindarajan, 1973). The ferrous species Mb and MbO_2 oxidise to ferric MetMb upon which the oxygenation ability is lost. MbO_2 is more stable to autoxidation compared with Mb (Govindarajan, 1973; Stryer, 1981). The reduction of Mb is not reversible, but it only becomes reversible in the prescence of a reductor, while MetMb is accumulated in the meat due to continous autoxidation in the inherent redox potential during storage. The development of MetMb at the meat surface depends essentially on the myoglobin autoxidation rate, enzymic MetMb reduction and oxygen consumption rate (Renerre, 1990).

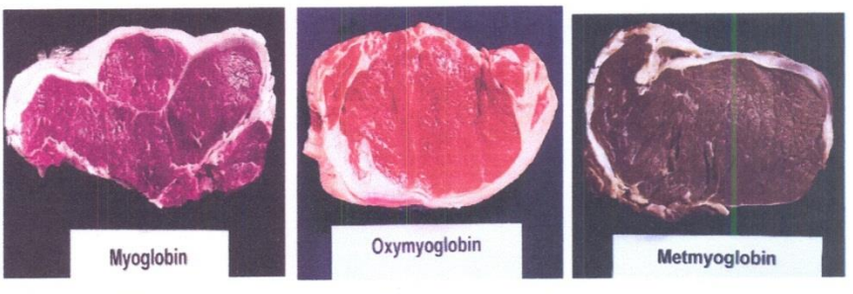


Plate 3: The various pigment species in meat sample

(Boles and Pegg, 2011)

2.4.4 Oxygenation

When meat is freshly cut, the myoglobin is in the purple reduced form Mb. On exposure to air, myoglobin rapidly and reversibly combines with oxygen to form the bright cherry-red MbO_2 (Ledward, 1992). The myoglobin oxygenation is a first order reversible reaction (Bevilacqua and Zaritzky, 1986). The meat surface

blooms to the bright cherry-red colour within minutes of exposure to air (Ledward, 1992) and with time, the small layer of Mb02 spreads downwards into the meat (Feldhusen *et al.*, 1994). The depth to which Mb02 diffuses depends on the activity of the oxygen-consuming enzymes, i.e. the oxygen consumption rate of the meat, the temperature and external oxygen pressure . (Ledward, 1992). MetMb reducing activity may also be involved. The oxygen diffuses through the aqueous environment and enters the hydrophobic heme crevice to occupy the sixth coordination site. Blooming is more efficient under conditions that increase oxygen solubility and discourage enzymic activity, i.e. at low temperatures and low pH values. Thus meat that has been aged for several weeks in vacuum prior to exposure to air blooms more rapidly and intensely than fresh meat owing to some loss of activity of the oxygen-consuming enzymes (Ledward, 1992), and formation of a deeper Mb02 layer (Feldhusen *et al.*, 1994).

2.4.5 Oxygen consumption

Oxygen uptake in *post rigor* muscle results from tissue respiration, reaction with heme pigments and dissolution into tissue fluids (DeVore and Solberg, 1974). *Post mortem* muscle is not inert, and mitochondria continue to metabolise oxygen (Lanari amd Cassens, 1991; Madavi and Carpenter, 1993; Tang *et al.*, 2005). Mitochondrial activity in *post mortem* muscle is enhanced by high pH values (Ashmore *et al.*, 1972; Bendall and Taylor, 1972) and high storage temperature (Bendall, 1972; Bendall and Taylor, 1972), which simultaneously results in higher oxygen consumption. Moreover, the oxygen consumption rate is influenced by muscle type due to differences in muscle metabolism as shown in studies on beef

(Lanari and Cassens, 1991; Madavi and Carpenter, 1993). However, as a consequence of respiration the oxygen consumption rate declines with time *post - mortem* in porcine and bovine muscle (Lanari and Cassens, 1991; Madavi and Carpenter, 1993; Tang *et al.*, 2005). This decline is due to loss in structural integrity of the mitochondria (Cheah and Cheah, 1971; Tang *et al*, 2005), which subsequently allows oxygen to penetrate further into the muscle (Morley, 1971; Feldhusen *et al.*, 1994; Millar *et al.*, 1994) and a more pronounced blooming (Feldhusen *et al.*, 1994; Zhu *et al.*, 2001; Lindahl *et al.*, 2005) that gives rise to a more red colour (Rosenvold and Andersen, 2003; Lindahl *et al.*, 2005).

2.5.0 Factors affecting meat colour stability

According to Robin *et al* (2008), colour stability can be influenced by a number of management factors along the meat supply chain from farm, to meat processing and retail display. The under listed are said to be factors affecting meat colour in a supply chain and good management approach achieves the best result for colour stability.

2.5.1 Muscle type

Colour of meat is (partly) determined by the oxygenation of myoglobin (Kiont *et al.*, 1998). Type I and HA fibres have greater myoglobin content (Essen-Gustaysson *et al.*, 1992) as well as a higher concentration of mitochondria compared with type IID fibres.

This means that meat with high percentages of oxidative fibres has a red colour.

Muscles can be classified as glycolytic (white) or oxidative (red) due to their dominating energy metabolism, which is based on the proportion of glycolytic and oxidative fibre types in the muscle (Cassens *et al.,* 1968; Beecher *et al.,* 1965). Glycolytic muscles require a rapid source of energy, and glycolysis is the predominant metabolic pathway used by these muscles. Glycogen and many of the enzymes related to glycolysis are abundant in these muscles. Oxidative muscles rely primarily on oxidative metabolism, which requires a large amount of myoglobin for oxygen storage. As compared with white fibres, the red muscle fibres tend to be smaller in diameter, richer in mitochondria, myoglobin and lipid, and more generously supplied with blood due to higher capillarisation (Foegeding *et al.,* 1996).

The shelf-life of meat is influenced by this intrinsic biochemical nature of different muscle types. This is important because different cuts contain different muscle types. Some cuts, such as the silverside, do not change in colour within a two-day display time. Other cuts such as topside and rump change colour dramatically within two days of slicing (from oxygenation to metmyoglobin will change by greater than 25%). In between these extremes are cuts such as loin, which are intermediate in colour stability. However under some circumstances, such as ageing for more than 3 weeks before slicing, cuts that are stable may become unstable in colour. Therefore some care is needed in using this broad classification to compare the colour stability of different cuts. In general terms, if a commercial cut is very red, the colour will be less stable and the more responsive the cut will be to management interventions designed to improve colour stability (Foegeding *et al.*, 1996).

2.5.2.0 Production factors

2.5.2.1 Vitamin E

Vitamin E supplementation can increase the brightness of meat bloom colour as well as the stability of the red colour during shelf display. Vitamin E is a powerful antioxidant whose natural concentration in pastures and crop stubbles decreases seasonally during the dry period. The appearance of brown on the meat surface is an oxidative process; therefore vitamin E concentration in the muscle has an influence on meat colour (Buys *et al.*, 2000).

2.5.2.2 Age

As lamb age increases a subtle change in meat colour occurs. Meat from "carryover" lambs tends to be darker, more intense, less stable and more variable in colour than meat from "sucker" lambs. These changes result from muscle pigment
(myoglobin) increasing with lamb age, but may also be due to changes in feed type as the seasons change (Robin *et al.*, 2008).

2.5.2.3 Genotype

Selection for growth rate and muscling has the potential to change the colour of lamb meat. Lambs selected for muscling may have lighter coloured meat, which is

more acceptable. Leg cuts from Merino lambs may be darker, less red in colour and less stable in colour during retail shelf display than those from crossbred lambs of the same age. However, this difference has not been seen with Merino loins (Robin *et al.*, 2008).

2.5.3.0 Meat processing factors

2.5.3.1 Electrical stimulation

Apart from improving meat tenderness, electrical stimulation has been shown to improve initial meat colour although its stability during extended retail display has been questioned. The effect of electrical stimulation depends to some extent on the type of system used, but a general effect of electrical stimulation is to make the bloom colour lighter and more attractive to consumers. New generation medium voltage systems generally have no effect on colour stability whilst high voltage systems may cause a small reduction in colour stability. However, these effects depend to some extent on the muscle type and other production and processing factors (Pommier, 1992; Powell *et al.*, 1996). Early application of stimulation, irrespective of type of stimulation, improved meat colour at 24 h, (Hwang and Thompson, 2003)

2.5.3.2 Primal packaging systems

Packaging meat as primal cuts in carbon dioxide causes meat to be redder in hue and more stable in colour compared to meat kept in carcass form exposed to air.

Packaging in carbon dioxide can also reduce the likelihood of electrical stimulation having a negative effect on colour stability (Buys, 2004).

2.5.3.3 Ageing period

Ageing meat to improve tenderness can reduce colour stability. This effect is seen for meat aged longer than 10 days and the maximum time suggested for ageing without substantial detrimental effects on colour stability is 20 days. If meat is to be aged for extended periods of time, carbon dioxide gas packaging should be considered and the vitamin E status of the animal should be known. Cuts that are normally stable in colour, such as the silverside, become very unstable in colour when aged for longer than three weeks prior to slicing for retail display (Robin *et al.*, 2008).

2.5.3.4 Chilling, freezing rate and hot boning

Rapid chilling can improve the colour stability of meat. Hot boning can cause meat to appear darker in colour compared to cold-boned meat. Meat that has been frozen tends to be less stable in colour than fresh meat (Warriss, 2010).

2.5.3.5 Acidity pH

An increase in glycolysis results from excessive excitement, starving and stress caused by ambient temperature, which in turn leads to high post-mortem pH values and consequently meat colour is influenced (Hwang and Thompson, 2003).

The ultimate pH of meat affects everything from its colour, tenderness and eating quality, to its storage life. The normal pH for beef and lamb is 5.4 to 5.6. Within this range the meat is a bright, attractive red colour and has good eating quality.

At a lower pH (below 5.3) meat will be pale and soft due to denaturation of myoglobin result in less desirable appearance and may be associated with other undesirable palatability characteristics in fresh meat, such as Pale, Soft and Exudative (PSE) pork, which is one of the largest economic burdens to the meat industry. The characteristics of PSE include undesirable colour, soft texture, and excessive purge. Pork carcasses that produce PSE product often have an accelerated rate of post-mortem metabolism, which can produce a combination of high temperature and low pH in muscle tissue. An increase in pH above about 5.8 may indicate an overall decrease in meat quality. High pH meat (pH more than 6) is dark with a slightly different odour and flavour. Meat becomes progressively less juicy as pH increases. High pH meat spoils rapidly

due to its different biochemical make-up (Sauerland, 2008).

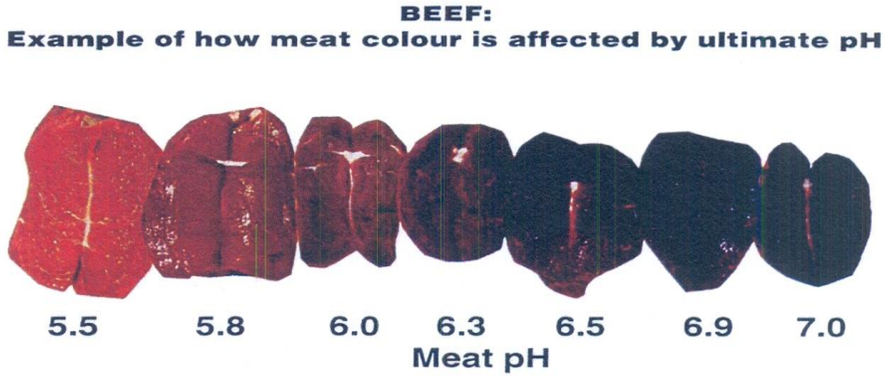
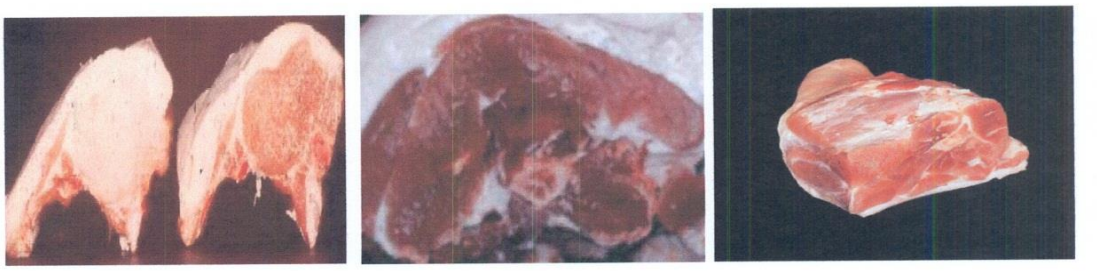


Plate 4: Effect of ultimate meat pH on the colour of beef

(Arie, 2011)



PSE pork DFD pork Normal pork

Plate 5: Effect of pH on PSE and DFD pork and normal
(MBG, 2007; FOA, 2008)

2
.5.4.0 Retail factors

2.5.4.1 Packaging

According to (Robin *et al.,* 2008) Modified atmosphere packaging (MAP) can improve colour stability. These systems involve packaging in an oxygen impermeable package that contains a carbon dioxide, nitrogen and oxygen gas mixture. Control of this gas mixture can influence the rate of oxidation of myoglobin. A study in Victoria has shown that MAP doubled the acceptable shelf-life of lamb meat.

2.5.4.2 Temperature

The rate of oxidation (browning) increases as the temperature of the display cabinet increases. Display cabinets should be kept as low as possible. Temperature fluctuations will also promote oxidation (Robin *et al.*, 2008).

2.5.4.3 Lighting

The structural properties of the muscle affect the reflectance of light from the meat surface and therefore its perceived paleness (Warriss, 2010). Light can speed the oxidation process so the more intense cabinet lighting is, the faster the meat will discolour (Robin *et al.*, 2008).

2.5.4.4 Bacterial Activity

Bacterial activity as a major factor in pigment changes in fresh prepackaged meat has been well established (Butler *et al.*, 1953; Costilow *et al.*, 1955). Neill (1925) reported that pneumococci, cell-free extracts of pneumococci, and sterile animal tissue extracts caused the reduction of methemoglobin to hemoglobin when molecular oxygen was excluded.

According to Robach and Costilow (1961) pure cultures of *Pseudomonas fluorescens, Pseudomonas aeruginosa,* two strains of *Pseudomonas geniculata, three Pseudomonas sp., Achromobacter liquefaciens, Flavobacterium rhenanus, L plantarum, and Saccharomyces cerevisiae* have effect on the surface colour of wrapped beef steaks. At room temperature, the yeast and all the bacteria tested except *L. plantarum* caused rapid colour changes. At 4 ± 1°C' the yeast had little

effect on colour but the aerobic bacteria tested were all active and pigment changed from red to brown and finally to purple (Robach and Costilow, 1961). Lawrie and Ledward (2006) reported that, oxygen availability aids meat spoilage micro-organisms to cause discolouration of fresh meat e.g. molds, aerobic bacteria and yeast.

CHAPTER THREE

MATERIALS AND METHODS

3.1 The study Area

The study was conducted in the Techiman Municipality of Brong Ahafo Region, which lies between longitudes 1˚49' East and 2˚30' West and latitude 8˚00' North and 7˚35' South Techiman Municipal Assembly (TMA, 2013). It shares common boundaries with Wenchi Municipality to the North-West, Kintampo South District to the North-East, Nkoranza South District to the South-East and Offinso-North District (Ashanti Region) to the South (TMA, 2013). Total land area is 669.7km^2 representing 1.69% of the surface area of Brong Ahafo Region (TMA, 2013). The Municipality has a population of 234,988 with an average growth rate of 3.84% per annum and a population density of over 351 persons/Km^2, Ghana Statistical Service (GSS, 2010). The Municipality experiences both semi-equatorial and tropical savanna climates, marked by moderate to heavy annual rainfall Meteorological Department (MD, 2009). The major rains start from April to July and the minor from September to October with mean annual rainfall ranging between 1660mm and 1260mm (MD, 2009). The dry season, which is highly pronounced in the Savanna zone, starts in November and lasts until March. The average monthly temperature is about 30˚C which occurs mostly between March and April while the lowest temperature (20˚C) occurs in August. The relative humidity of 75-80% is recorded in the rainy season while 70-72% for the rest of the year (MD, 2009). There are three main vegetation zones, namely, the guinea-savanna woodland, in the North-West, the semi-deciduous zone in the South and

the transitional zone stretches from the South-East and west up to the North of the Municipality (TMA, 2013).

3.2.0 Sample collection

3.2.1 Butchery/ Retail sites selection

Fifteen (15) butcheries (meat retail shops) receiving fresh beef carcasses from the Techiman Municipality abattoir were purposively selected. These are the areas that mostly supply beef each day to consumers within the Municipality; they include the following markets; Main, Kenten, Takofiano, Hansua, Dwomor, Zongo, Nana Abena, Ahenfie, Sansama Junction, Zongo-Tamale Station, Abanim, Site, Anyenabrim and Brigade as well as the Abattoir. The butcheries were among those registered with the Local Authority and are in possession of certificates of acceptability in terms of Municipal Regulation on health and food inspectorate. The butcheries were visited after the carcasses have been delivered to the retail shops and were randomly selected.

3.2.2 Location, duration, sample collection and transport

A total of 240 samples, made up of 60 each of beef, table, knife and apron samples were randomly collected aseptically using sterile cotton swabs and sterile metal frame of 10 cm^2 from 15 retails points in Techiman Municipality. The portions swabbed were; beef- any portion of the flesh of fresh beef that was

available, knife — the two surfaces, apron- pockets sides, just around the belly and area stained with splash of blood and table- the top surfaces with crevices.

The study was carried out between April and November 2014. The samples collected were stored in an ice chest under 4˚C, transported to laboratory under aseptic conditions and analysed immediately on arrival for the presence of *Escherichia coli* and *Salmonella*.

3.3.3 Sampling procedures

- Sterile cotton swabs were removed from their containers, care was taken to ensure that throughout the handling of the swabs, the fingers did not touch the cotton tip.
- The sterile template was pressed against the surface to be sampled.
- The swabs were vigorously rubbed over the above mentioned areas for better absorption or soaking up of the bacteria and rotated to bring the whole area of the cotton tip into contact with the surface.
- The inoculated swabs were placed into the tube soon as after rubbing.

3.3.4 Handling, storage and transportation of samples

Samples were stored in an ice chest under 0-4°C and transported to the laboratory for examination immediately on arrival.

3.3.5 Microbiological examination

The samples were analysed at the Spanish Microbiological/Biotechnology Laboratory of University for Development Studies, Nyankpala, Ghana.

3.3.6 Media and reagents used for isolation of *Escherichia coli* and *Salmonella*

Both selective and differential media were used for *E. coli* and *Salmonella* isolation in the study. All media used were purchased from Oxoid, UK. They include; Peptone water, Levine Eosin Methylene Blue (LEMB), Rappaport Vassiliadis (RV) broth, Selenite-Cystine (SC) Broth, Brilliant green Agar (BGA), Xylose Lysine Deoxycholate agar (XLD), Triple Sugar Iron (TSI) Agar, Lysine Iron Agar (LIA), Nutrient Agar (NA), Simmons Citrate agar, MaConkey, Gram Stain Reagent and *Salmonella* and *E. coli* latex agglutination test kits. The composition and preparation of the media is presented in the appendix.

3.3.7 Isolation, confirmation and identification of *Escherichia coli* in fresh beef and related samples

The samples on arrival were cultured into 10m1 of peptone buffered water and incubated at 37°C for 24hours. After which 0.1m1 of the culture, was streaked on LEMB agar with the use of metal loop and incubated at 37°C for another 24 hours. Presumptive *E. coli* colonies appear as dark centred and flat, with or without metallic sheen. Such colonies were isolated and purified on Nutrient agar

and incubated at 37°C for 24hours. It was identified and confirmed using Gram stain, growth on MacConkey agar, Brilliant green bile broth, Simmons Citrate agar and Latex agglutination test kit.

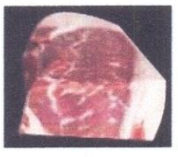
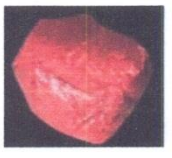
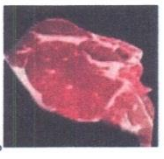
3.3.8 Isolation, confirmation and identification of *Salmonella* in fresh beef and related samples

Isolation and identification of *Salmonella* from various fresh beef and related samples was done by culturing the cotton swabs in 10m1 of peptone buffered water and incubated for 24 hours for pre-enrichment. After pre-enrichment, 1 ml portions were transferred to 10m1 of Rappaport Vassiliadis (RV) broth and selinite cysteine (SC) broth and incubated at 42°C and 32°C respectively for 24 hours to 48 hours. After which 0.1m1 of the culture, was streaked on xylose lysine deoxycholate (XLD) and brilliant green agars by using metal loop. The plates were incubated at 37°C for 24 to 48 hours. Presumptive single isolated colonies of *Salmonella* were picked, purified, on nutrient agar (NA). Presumptive *Salmonella* isolates were subjected to confirmational and biochemical test such as triple sugar iron (TSI) agar and lysine iron agar (LIA) test. Latex agglutination test and Gram stain.

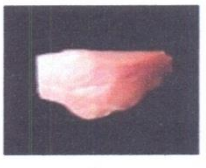
3.4.0 Assessment of aesthetic quality 3.4.1 Photographing

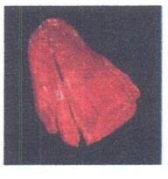
Similar sizes of the fresh beef samples, on which the swabs were taken on display, were also taken immediately after swabbing, kept in airtight polystyrene bags and sent to photo studio within five minutes.

Photographing was done immediately on arrival using a modified method of Bocard *et al.* (1981) with Olympus DP21 digital camera at Professional photo studio in Techiman. The camera was set up with the lens aligned 50cm from the meat surface and the focus set at focal length of 50cm. The images were saved into JPEG file format. The photographs were printed on black background. Finally eight pictures were selected and stuck on cardboard and labeled A-H. They were then compared with colour chart that were described as;


A-Scarlet,

B- scarlet red,

C- bright cherry red,


D-Magenta,

E-crimson,

F- pale white,


G- Dark purplr/ purple magenta and

H- red.

Two hundred and forty (240) beef consumers panels were sampled at 15 beef retail points in August 2014. The panelists were pretested to distinguish seven colour cards of the same with different shades to screen out people having problems with colour blindness. People, who were under the age of eighteen and those obviously in a rush, were excluded from evaluating the beef colour using the photographs.

Subjective sensory (colour) assessments were performed using the photographs as photographic scales to determine consumers' acceptability of fresh beef colour as its aesthetic quality by a ranking test (British Standard Institution (BSI), 1980) where each consumer was asked to rank samples in order of preference of each colour (one (1) corresponds to the highest preference and eight (8) the lowest preference) as reported by American Meat Science Association (AMSA), 1991):

1: Extremely desirable colour

2: Very desirable colour

3: Moderately desirable colour

4: Slightly desirable colour

5: Slightly undesirable colour

6: Moderately undesirable colour

7: Very undesirable colour

8: Extremely undesirable colour

Panelists were asked to comment on the statements below

(i) Perceptions on the colour of fresh beef or

(ii) Expectation when purchasing fresh beef.

3.5.0 Data analyses

Microbial qualities (prevalent data) were calculated in proportions and the results presented in percentages. That is Sample positive ÷ Sample tested x 100. Further statistical analyses were done using Generalized Linear Models (GLM) for pairwise comparisons of estimated marginal means based on the original scale of dependent variable of samples and retail shops.

Consumer colour preferences were analysed by Analyses of Variance (ANOVA) using 'GENSTAT' l0.DE 4th edition, calculated in proportions, ranked and results presented in statistical tables and figures.

CHAPTER FOUR RESULTS

INTRODUCTION

The results of this study are categorized in two folds. The *Salmonella* and *E. coli* prevalence and consumer preference for fresh beef colour (aesthetic) assessment both from fifteen different retail points in Techiman, Ghana.

4.1 *Salmonella* and *Escherichia coli* prevalence in fresh beef, table, knife and apron samples

From the 15 retail points visited in this study, *Salmonella* and *Escherichia coli* were detected in the fresh beef, table, knife, and apron and the results are presented in Tables 3-10. Overall prevalence of *Salmonella was* 57.1% (Table 3). The specific prevalence within different retail points was 93.75%, for Kenten market, Main market and slaughter house. Anyinabrem recorded 87.5%. Site, Hansua market and Brigade all recorded 81.3% prevalence, Nana Abena market 68.8%, Dwormor market 62.5%, Takofiano market 37.0%, Ahenfie market 25%, Abanim and Zongo-Tamale station recorded a prevalence of 18.8%, Zongo market and Sansama junction with the least prevalence of 12.0% and 6.3% respectively. From the total of 240 samples tested, fresh beef recorded 75% overall prevalence of *Salmonella* as the highest; 60% positive for table samples, 55% for knife samples and apron samples had the least prevalence of 33.3%. The difference in the prevalence of *Salmonella in* meat, knife, table and apron were highly significant ($p>0.001$) between apron and beef samples.

There was also significant differences (p>0.05) between apron, knife and tables. But there was no significance difference between positive isolates of *Salmonella* on beef, table and knife samples. There were significant differences (p>0.05) in the prevalence from the retail markets of Abanim, Anyinabrem, Brigade, Hansua market, Kenten market, Main market, site and slaughter house. Abanim, Dwomor market and Nana Abena market were slightly significant (p>0.05). However, Zongo market, Zongo Tamale station, Ahenfie market and Sansama Junction did not differ significantly (table 9). Ahenfie market was significantly higher (p>0.05) among all except Abanim, Sansama junction, Takofiano market, Zongo Market and Zongo-Tamale Station. Anyinabrem was significantly different (p>0.05) from Abanim, Ahenfie market, Sansama junction, Takofiano market, Zongo Market and Zongo-Tamale Station. There were highly significant differences (p>0.05) (table 9) between Brigade, Abanim, Ahenfie market, Sansama junction, Takofiano market, Zongo Market and Zongo-Tamale Station.

The overall prevalence of *E. coli* was 79.2%. The highest prevalence of *E. coli* occurred in the fresh beef and table samples 91.7%, followed by the knife 85.0% and apron with the least prevalence 48.30%. The beef retail points visited at Hansua market emerged as the highest, recording 100% prevalence. Anyinabrem, Kenten market and Slaughter house recorded occurrence of 93.8%, while Main, Nana Abena and Ahenfie markets all recorded incidence of 87.5%. Among all Abanim and Sansama juction recorded 50% and 37.5% respectively as the least incidence (Table 5).

Fifty-two point five percent of the samples tested were both positive for *Salmonella* and *Escherichia coli,* of which beef sample was the highest with 70% and apron was the least (25%). The means of positive isolates of *E coli* indicated that, apron differs significantly ($p>0.05$) from knife, beef and table samples. But there was no significant difference between beef, knife and table samples. Prevalence of *E. coli* isolates at Abanim retail point varies significantly ($p>0.05$) from that of Ahenfie market, Anyinabrem, Brigade, Dwomor market, Hansua market, Kentent market, Main market, Site and Slaughter house. There were no significant differences between Abanim, Sansama junction, Takofiano market, Zongo market and Zongo-Tamale station. There were no significant differences between Ahenfie market and all the retail points except Abanim and Sansama junction. Abanim and Sansama junction were significantly different ($p>0.05$) from all except Zongo market and Abanim (table 10).

Table 3: Prevalence of *Salmonella* at different beef sales points in Techiman

Location	Number tested	Number positive	% of prevalence
Kenten market	16	15	93.75
Main market	16	15	93.75
Slaughter house	16	14	93.33
Anyinabrem	16	14	93.33
Site	16	13	81.25
Hansua market	16	13	81.25
Brigade	16	13	81.25
Nana Abena market	16	11	68.75
Dwomor market	16	10	62.5
Takofiano market	16	6	37.5
Ahenfie market	16	4	25
Abanirn	16	3	18.75
Zongo-Tamale Station	16	3	18.75
Zongo market	16	2	12.5
Sansama junction	16	1	6.25
Overall	240	137	57.1

*p>0.05

Table 4: Prevalence of *Salmonella* in beef, table knife and apron samples

Sample	No. of samples tested	Number positive	% of prevalence
Beef	60	45	75
Table	60	36	60
Apron	60	20	33.33
Overall	240	137	57.1.

*P>0.05

Table 5: Prevalence of *Escherichia coli* at different beef sales points in Techiman

Location	Number	Number positive	% of prevalen
Hansua	16	16	100
Anyinab	16	15	93.8
Kenten	16	15	93.8
Slaught	16	15	93.8
Main	16	14	87.5
Nana	16	14	87.5
Ahenfie	16	14	87.5
Brigade	16	13	81.3
Dwomo	16	13	81.3
Site	16	13	81.3
Takofia	16	12	75
Zongo-Tamale	16	12	75
Zongo	16	10	62.5
Abanim	16	8	50
Sansam	16	6	37.5
Overall	**240**	**190**	**79.2**

*p>0.05

Table 6: Prevalence of *Escherichia con* in beef, table, knife and apron samples

Samples	Number of samples	Number positive	% of prevalence
Beef	60	55	91.7
Table	60	55	91.7
Knife	60	51	85
Apron	60	29	48
Overall	**240**	**190**	**79.2**

*p>0.05

Table 7: Percentage of both *Salmonella* and *Escherichia con* prevalence in sample tested

Sample	Number tested	Number positive	% of Prevalence
Beef	60	42	70
Table	60	35	58.3
Knife	60	34	56.7
Apron	60	15	25
Overall	**240**	**126**	**52.5**

*p>0.05

Table 8: Percentage of retail shops that recorded prevalence of both *Salmonella* and *Escherichia coli*

Location	Beef	Table	Knife	Apron	% of prevalence
Kenten market	4	3	4	3	87.5
Main market	4	3	3	3	81.2
Slaughter house	4	4	3	3	87.5
Anyinabrem	3	4	4	2	81.25
Site	3	4	4	0	68.75
Hansua market	4	3	3	3	81.25
Brigade	4	3	3	1	68.75
NanaAbena market	4	4	3	0	68.75
Dwomor market	4	4	2	0	62.5
Takofiano market	2	1	3	0	37.5
Ahenfie market	2	1	1	0	25
Abanim	3	0	0	0	18.75
Zongo -Tamale Station	1	1	0	0	12.5
Zongo market	0	0	1	0	6.25
Sansama junction	0	0	0	0	0
Overall total	42	35	34	15	126
Percentages (%)	70%	58.3	56.7	25	52.5

*P>0.05

Table 9: Pairwise comparisons of estimated marginal means based on the original scale of dependent variable *Salmonella* samples (p>0.05)

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error		Sig.
Apron	Knife	.27[a]	.088	1	.002
	Beef	.42[a]	083	1	.000
	Table	.27[a]	.088	1	.002
Knife	Apron	-.27'	.088	1	.002
	Beef	.15	.084	1	.076
	Table	.00	.089	1	1.000
Beef	Apron	-.42[a]	.083	1	.000
	Knife	-.15	.084	1	.076
	Table	-.15	.084	1	.076
Table	Apron	-.27'	.088	1	.002
	Knife	.00	.089	1	1.000
	Beef	.15	.084	1	.076.

*Means with the same superscript are significantly different

Table 10: Pairwise comparisons of estimated marginal means based on the original scale of dependent variable Bacteria (*Escherichia coli*) (p>0.05)

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	df	Sig.
Apron	Knife	.37[a]	.079	1	.000
	Beef	.43[a]	.074	1	.000
	Table	.43[a]	.074	1	.000
Knife	Apron	-.37[a]	.079	1	.000
	Beef	.07	.058	1	.253
	Table	.07	.058	1	.253
Beef	Apron	-.43[a]	.074	1	.000
	Knife	-.07	.058	1	.253
	Table	.00	.050	1	1.000
Table	Apron	-.43[a]	.074	1	.000
	Knife	-.07	.058	1	.253
	Beef	.00	.050	1	1.000

*Means with the same superscript are significantly different

4.2 Consumer preference for fresh beef colour (aesthetic) assessment

The panelists' scores were averaged for statistical analysis using standard analysis of variance techniques and results presented in tables and statistical figures. The comments of expectation or perception for the choice of a particular beef product by correspondents were sought for, calculated in proportions and presented in percentages.

Table 11: Ranking of consumer preferences for fresh beef colour

Beef colour sample	Means of colour preferences	Ranking
A	3.2^{c}	Moderately desirable
B	3.6^{d}	Moderately desirable
C	1.3^{a}	Extremely desirable
D	2.7^{b}	Very desirable
E	5.4^{e}	Slightly undesirable
F	7^{f}	Very undesirable
G	7.3^{g}	Very undesirable
H	5.5^{e}	Slightly undesirable

P. Value: 0.001

SED: 0.1031 *Means within column with different superscripts are significantly different

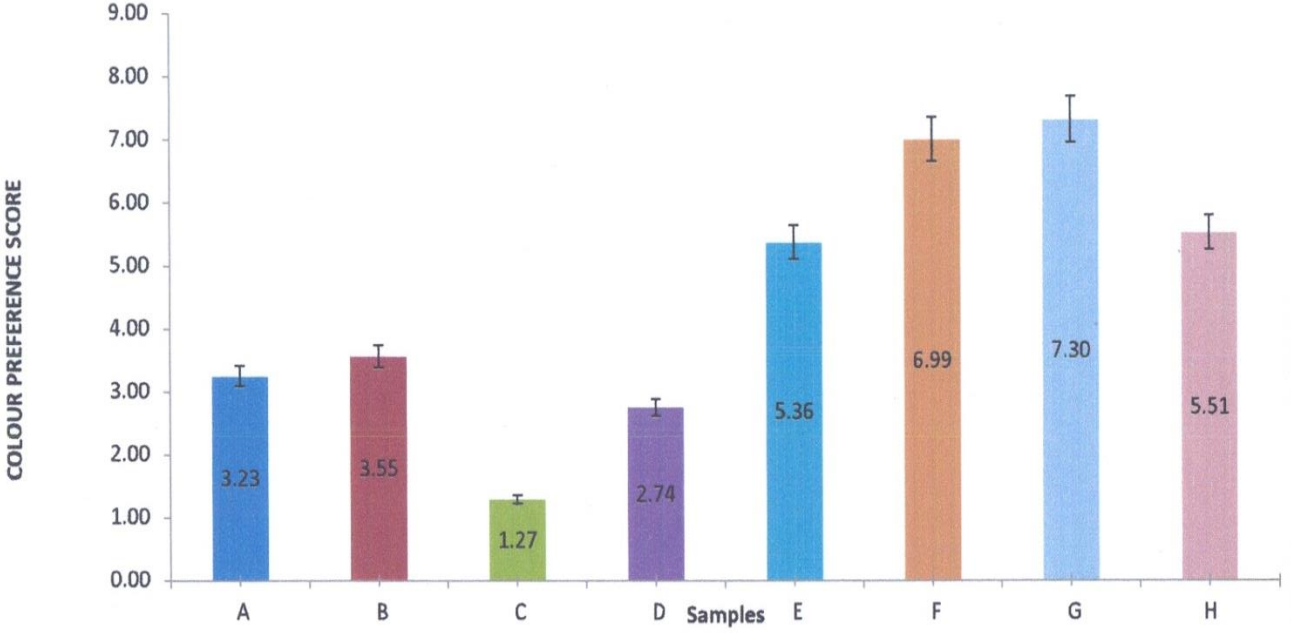


Figure 2: Consumer preferences of fresh beef colour

*A-H ⟶ Photograhs of fresh beef samples and colour description

*A-Scarlet, B-Scarlet red, C-Bright cherry red, D-Magenta, E-Crimson, F-Pale white, G-Dark purple/purple magenta and H-Red.

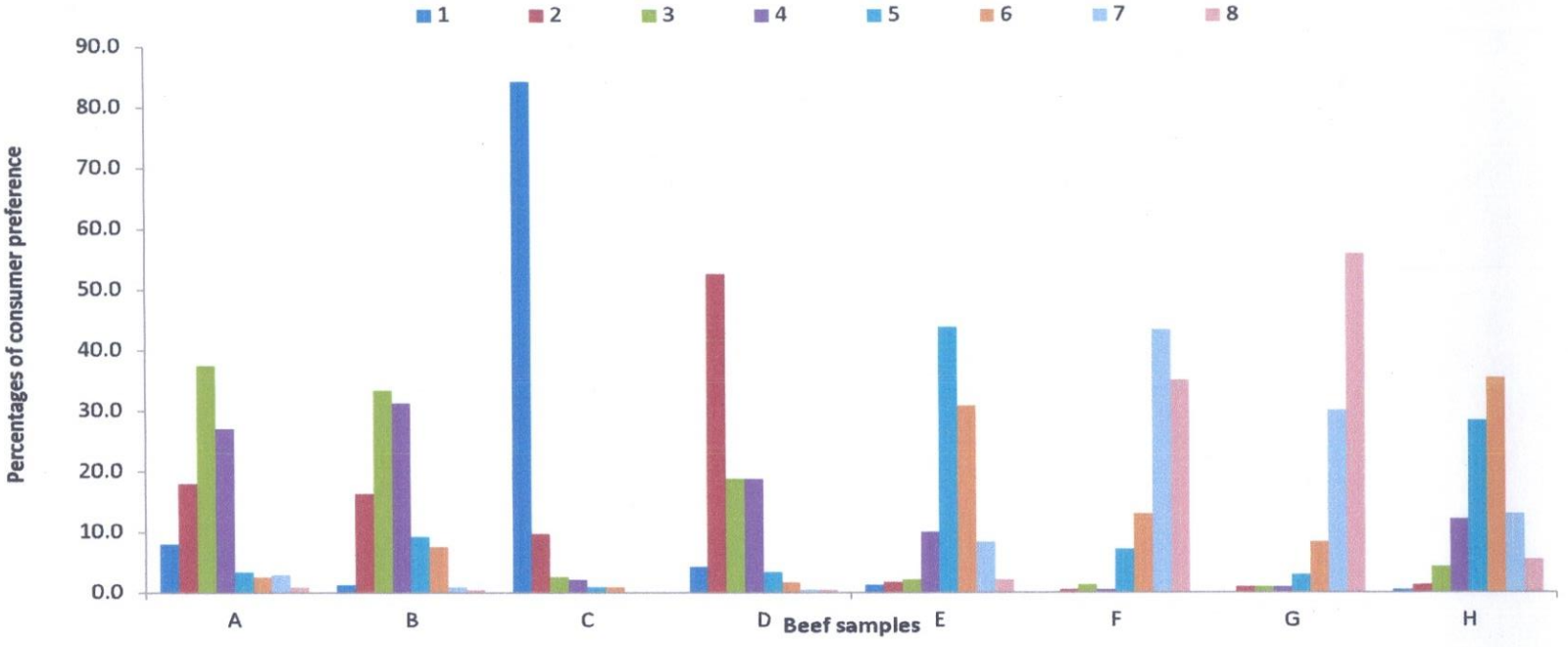


Figure 3: Consumer preferences for fresh beef colour in Techiman Municipality

1-Extremely desirable colour, 2-Very desirable colour, 3-Moderately desirable colour, 4-Slightly desirable colour, 5-Slightly undesirable colour, 6 - Moderately undesirable colour , 7- Very undesirable colour and 8 - Extremely undesirable colour

Table: 12 Expectation of correspondents' preferences for a particular beef product when purchasing

Consumer expectation	No. of consumers	% Score	Ranking
Fresh Bright colour	121	50.4	1
Quantity (size) of serving	5	2.1	6
Wholesomeness	81	33.8	2
Trimmings /Marbling/fats	8	3.3	4
Lean cuts	10	4.2	3
Part of cut of the beef/ portion	6	2.5	5
Price/cost	3	1.3	8
Relation established	2	0.8	9
Flesh/boneless or bone-in	4	1.6	7

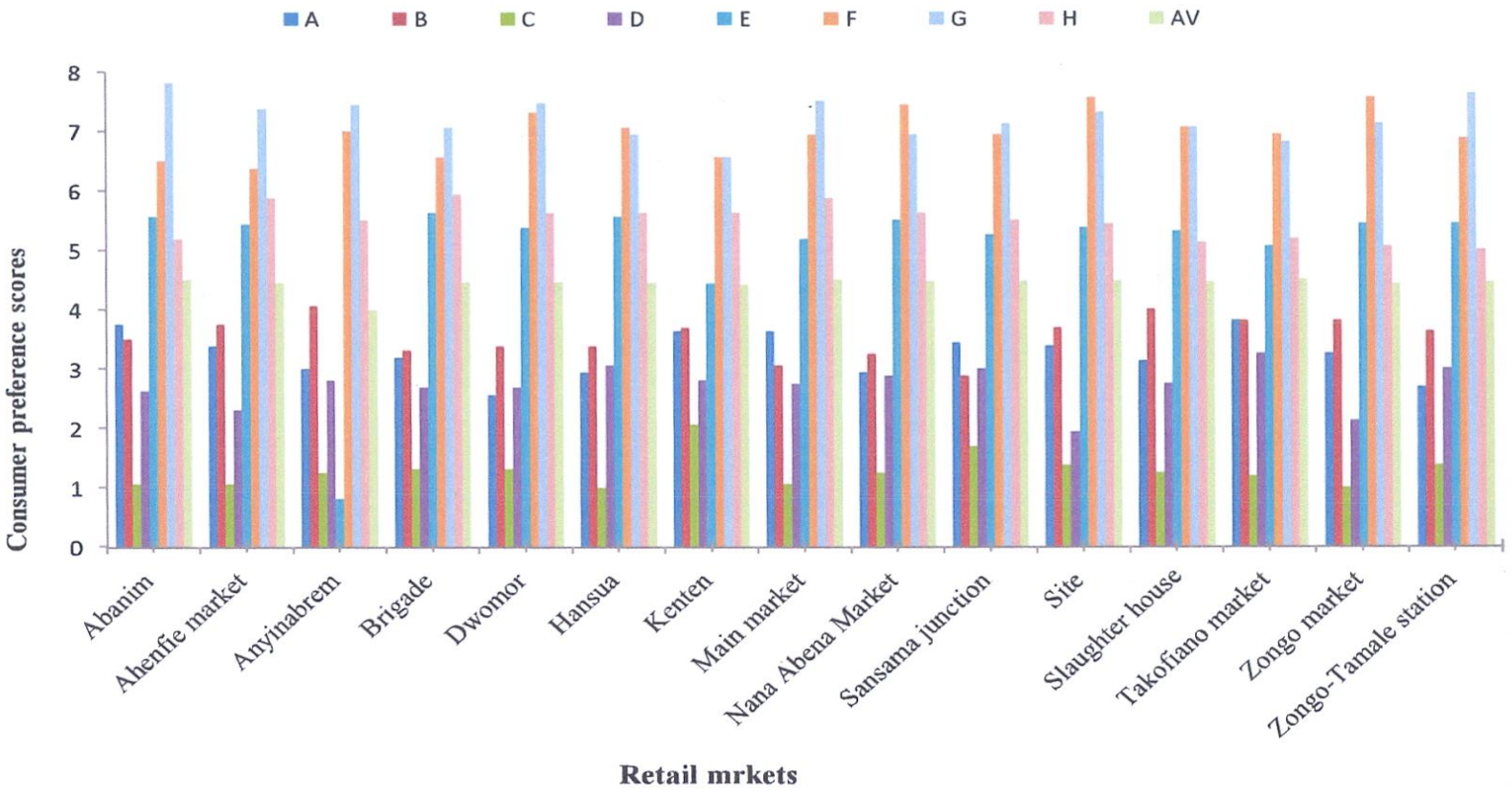


Figure: 4 Mean distribution of consumer preference at various retail points

* Colour description of beef samples: A- scarlet, B- scarlet red, C- Bright cherry red, D- magenta, E- Crimson, F- pale white, G- Dark purple/purple magenta and H- red

4.3 Aesthetic quality and consumers attitudes towards fresh beef colour

Colour influenced the purchase of beef for the majority of the 240 participants that were interviewed (Table 11). A significant difference ($p<0.001$) was recorded between all fresh beef colour samples except E (Crimson) and H (Red). Consumer scored sample C (Bright cherry red) 84.2% as extremely desirable, sample D (Magenta) 52.5% as very desirable, samples A and B (Scarlet and Scarlet red) 37.5%, 33.3% respectively as moderately desirable. Whiles samples F (Pale white), G (Dark purple/purple magenta) H (Red) and E (Crimson) were scored 0.00 %, 0.4%, 1.25% described as extremely undesirable rather scoring at 55.8%, 43.8, 43.3% and 35.4% for sample G - extremely undesirable, F-very undesirable, H-moderately undesirable and E-slightly undesirable.

The individual retail points showed no difference to that of general consumer preference, thus all the consumers at various retail points described sample C as extremely desirable colour except Kenten market which scored it as very desirable colour but failed to choose any of the samples as very extremely desirable. With the exception of Hansua and Takofiano markets scoring sample D as moderately desirable colour the rest of retails points accepted it as very desirable colour. Apart from Dwomor and Hansua markets all the retail markets scored sample A as moderately desirable colour. Most (12) of the retail markets described B as moderately desirable colour while Sansama junction, Anyinabrem and Slaughter house indicated that sample B was very desirable and slightly desirable colour respectively. The samples most retail markets discriminated against as slightly undesirable colours were H and E while F and G which were

said to be moderately undesirable and very undesirable colour by most of the consumers.

4.4 Consumers' expectations or perception on fresh beef colour at the point of purchase.

Consumers' expectations or comments at the point of purchase of fresh beef were sought for (table 12). Fifty point four (50.4%) of the panelists mentioned fresh bright colour as the highest expectation hence ranked first. Wholesomeness was ranked next with 33.8%. Lean cut ranked third 4.2%. However, the least intents at the point of purchase of fresh beef were the price/cost of the beef and the relationship established between the costumers and retailers 1.3% and 0.8% respectively.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of *Salmonella* and *Escherichia coli* in fresh beef, table, knife and apron samples

The high prevalence of *Salmonella* and *Escherichia coli* in fresh beef and related samples indicated that the meat samples were contaminated. Microorganisms can easily be introduced either in the pre or post processing stages of meat
(Johanson, 1983). Similar account was given by Enabulele and Uraih (2009) who reported *E. coli* prevalence rate to be 85.65% in a study with the fresh meat samples from abattoir and traditional open market each recording 100% *E. coli* prevalence.

It was obvious that, the prevalence of *Salmonella* and *E. coli* were high in the samples examined. Thus healthy cattle like other animals carry *Salmonella* and *E. coli* in their gastro-intestinal tract and consequently during dressing, evisceration and retail cutting the samples could be contaminated. There could also be cross-contamination as a result of used one knife throughout dressing as well as many men working on one carcass and for all other related activities. Again a single machete was being used in sharpening other knives, and to do other things. The source of water (dug well) for washing the carcass, working on bare cemented floor, poor condition of vehicles for transporting the meat, piling of different retail cut from different carcasses as well as offal together, the environment of retail display and the crevices on the tables at the retail points can harbour this organism and consequently cross-contamination can occur. Some *Salmonella* and *E. coli*

found in the apron could probable come from the beef, tables and hands of the retailers because the pocket sides of the aprons where they frequently keep their monies during retailing, the part close to the table were areas soiled with splashed blood and meat particles. This might have come from contaminated hands, beef and tables during retail. Processing procedures and retailing therefore expose aprons to *Salmonella* and *E. coli* and can cause contaminations or cross-contaminations to beef and other food products. Though further isolation was not done for the pathogenic strains but *Salmonella Enteritidis* and *diarrheagenic E. coli* could be present in the beef samples and subsequently a threat to public health. Again the prevalence of *E. coli* in the beef samples is an indication that, the beef were contaminated with faecal material since *E. coli* are used as surrogate indicator in food for faecal contamination (Clarence *et al.*, 2009). Hence the processing, transportation, retailing and other post slaughter handling are done under unhygienic conditions.

It is likely that the observed increase in fecal bacteria is due to problem associated with skinning on the floor and the fleece coming into contact with the surface of carcass (Ozlem, 2005). Chaubey *et al.* (2004) enumerated the coliform in majority of beef samples and suggested that raw beef and beef products should be handled under strict hygienic condition and stored in cool places to avoid contamination and safe guard the health of consumers. Contrary to this, the practice in Techiman is that fresh beef is displayed in the open air whereby all gases and flies have access to the meat and only the left over is kept under cold storage.

During the study, it was observed that, personal hygiene is hardly practiced, for instance most retailers leave their apron hang on board and nails in their shop unwashed after work.

There was no single retail point with water, soap, towel and sterilizer for hand washing and sterilising knife respectively. At the slaughter house it was realised that only a single knife was being used for slaughtering without sterilising and after each slaughter, the knife is pushed into its scabbard.

Apart from Main, Zongo and Nana Abena markets that were built with concrete blocks and fixed nets, sanitary practices were not strictly practice as there were no hand washing bowls, tables and chopping boards were not well washed, knives were not sterilized between cuts of beef, no wearing of nose and mouth mask etc. This could be the result of high contamination Sansama junction and Anyinabrem were next retail shops built with metal plates, but at Sansama junction, the tables were made with formica which ensure easy cleaning and no crevices for filth, this could be the result of least contamination of samples with both bacteria as compare to shops built with wooden structures and old sieve nets and wooden tables with crevices that were choked with debris. Retailers at Kenten market displays beef in the open with no protection from house flies, dust and gases. This could be the results of higher prevalence of both pathogens at Kenten market.

The high prevalence could also be from the fleece of cattle coming in contact with the carcass surfaces during hide removal (Bell *et al.*, 1993). The finding of this study is a reflection of the unhygienic practices of meat processing as reported by Singleton, (1995), Frazier and Westhoff (2004) and Bhandare *et al.* (2007) in the developing countries. It has been observed that the inner tissues of healthy animals are sterile, however, contamination comes from external sources during

bleeding, handling and processing. During bleeding, skinning and cutting, the main sources of microorganisms are the exterior of the animal which includes the hide, hooves and hair and the numbers and many kinds of microorganisms from the soil, wash water, feed and manure, as well as its natural surface flora and the intestinal contents which contain the intestinal organisms (Lawrie, 1984). Knives, environment of the abattoir, slaughter-slabs, hands and clothing of the workers and the physical facilities can serve as intermediate sources of contaminant (Lawrie, 1984). It has also been shown that during handling, contamination comes from carts, boxes or other containers, other contaminated meat, air and personnel. This resulted in the increase in the prevalence of the *Salmonella* and *E. coli on the* fresh beef and related samples (Lawrie, 1984). Retail cut could also contain greater microbial contamination due to large exposed surface area, more readily available water, nutrient and greater oxygen penetration available (Forest *et al.*, 1985). Hence, smaller retail cuts displayed are conducive for microbial growth and proliferation which leads to spoilage of the meat (Agnes, 1995). The fresh beef sold to the public in open retail markets is grossly contaminated with coliform bacteria as well as other bacteria and fungi. This work has revealed that the fresh beef sold in Techiman retails are contaminated by Gram negative bacteria. The bacteria isolated were *Salmonella* and *Escherichia coli*. These organisms isolated are in line with the work of Turtura (1991), Adak *et al.* (2005) and Clarence *et al.* (2009). They reported that Gram negative bacteria account for approximately 69% of the cases of bacterial food-borne diseases. The presence of these organisms in the fresh beef and related samples is indicative of public health hazard and gives a signal of the possible occurrence of food borne infection.

Though the pathogenicity and virulence strains were not determined, it could be possible that, these beef could be a viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards.

Escherichia coil are an enteric organism and its presence is an indication of faecal contamination of the samples (Clarence *et al.,* 2009). This may be attributed to improper sanitary conditions during processing of the meat, from the water supply, unsterilized utensils and contamination by flies (Brooks *et al.,* 2004). The presence of *Salmonella is as* a result of improper handling by butchers of meat transported to markets, storage, flies and environment. The handlers must observe strict hygienic measures so that they may not serve as source of contamination of microorganisms and fecal contamination of fresh meats and other meat products. It is known that hygienic cooking processes will greatly reduce the organisms to safety level. Thorough cooking as well as hygiene in order to prevent contamination of food eaten raw is also important (Amann *et al.,* 1995). It is therefore necessary that fresh beef for consumption should be adequately cooked before consumption.

5.2 Aesthetic quality and consumers attitudes towards fresh beef colour

Colour influenced the purchase of beef for the majority of the 240 participants that were interviewed (table 11 and 12). Beef consumers are more discerning and much particular about choice of products at the retail points with particular expectation to some attributes of the fresh beef. Among all sensory attributes of meat, 50.4% of the consumers indicated colour as purchasing intent.

Similar accounts were reported by Bekhit *et al.* (2005); Mancicni and Hunt (2005) that

deemed unacceptable, all other sensory attributes lose their significance to consumers and their purchasing decisions are negatively influenced (McKenna *et al.*, 2005). The choice of the consumers upheld the views of Mancini and Hunt (2005) and Brewer (1991) that, when a product does not meet 'colour expectations' it is a simple decision to consider a product unacceptable. Colour change is closely associated with spontaneous autoxidation of Mb (Trout, 2003) since Mb is the primary pigment associated with meat colour. In fresh meat Mb occurs in several forms: the most important is deoxymyoglobin (DMb), oxymyoglobin (0Mb) and metnlyoglobin (MMb). The oxygenated form of Mb (0Mb) is responsible for the bright-red color while the oxidised form (MMb) is responsible for browning (Bekhit *et al.*, 2001).

Discolouration is the result of oxidation Fe^{2+} iron into the Fe^{3+} iron and MMb formation, when meat becomes gray- red (brown). MMb formation depends on numerous factors, including the partial pressure of oxygen, temperature, pH, meat reduction activity and the presence and growth of microorganisms.

Reasons for preference for a particular beef product by the participants agreed to that of Warriss (2010) who reported that quality can be categorized into two main types as functional quality which refers to desirable attributes in the product and conformance quality. For instance consumers might want red meat to be tender, red coloured and chicken to have good flavour. Conformance quality which is, producing products that meet the consumer's specification. For example size of a portion of beef steak or chicken breast or fat to be of a given size or weight.

However, both are important because no one wants chicken breast, beef or pork that are exactly the right weight and size but have poor colour, flavour or texture and detrimental to their health. This is also affirmed by Chambers and Bowers (1993) that, essential stimulus such as colour, marbling fat etc. are known to carry more weight when consumers form meat quality expectations than extrinsic cues (packaging, number of servings etc.). Therefore sensory properties of a product must be entirely satisfied before other quality dimensions become pertinent.

Expectation or perceptions of consumers at the point of purchasing a particular beef product implied that, no matter how retailers entice the consumers, they will still refuse their beef product if it does not meet their colour expectation. Also consumers will of course buy a beef product as far as it meets their expectation without considering the price. However this is contrary to Rani (2012) indicated that price was one of the major factors affecting the purchasing decisions of consumers. Mpofu (2011) stated three categories of consumers such as high class, middle *class* and those living under the poverty line. Consumers classified as high class can afford to buy everything, even if a product is at a higher price. Their purchases are not determined by the amount of cash available. Middle class consumers are at least able to afford to purchase what they want, though they might not afford some expensive products. Consumers living under the poverty line are those whose majorities of purchases are determined by the amount of disposable cash available, such that most of the expensive products they cannot afford hence will mostly consider prices.

5.3 Effects of fresh beef handling on aesthetic quality at retails shops in Techiman

According to Robin *et al.* (2008), colour stability can be influenced by a number of management factors along the meat supply chain from farm, to meat processing and retail display.

Buys (2004) reported that, packaging meats as primal cuts in carbon dioxide causes meat to be redder in hue and more stable in colour compared to meat exposed to air.

However, this study reveals that, the retail shops do not practice any packaging systems before display, the beef are just put on tables and exposed to air that could cause most discolourations.

The structural properties of the muscle affect the reflectance of light from the meat surface and therefore its perceived paleness (Warriss, 2010). Light can speed the oxidation process so the more intense lighting is, the faster the meat will discolour (Robin *et al.*, 2008).

The high prevalence of microbes on the meat makes it brown and subsequently green. This made the consumers reject meat colour sample E. Butler *et al.* (1953) and Costilow *et al.* (1955) reported that, bacterial activity is a major factor in pigment changes in fresh prepackaged meat. Robach and Costilow (1961) indicated that, pure cultures of *Pseudomonas spp, Achromobacter liquefaciens,*

Flavobacterium rhenanus, L. plantarum, and Saccharomyces cerevisiae have effect on the surface colour of beef steaks. Sansama junction recorded the least prevalence of both *Salmonella* and *Escherichia coli* on the same sample tested hence; meat colours appeared most attractive to consumers and were ranked as extremely desirable.

The study revealed that the butchery practice of hot boning resulted in beef darker and were least preferred by the panelists. Rapid chilling can improve the colour stability of meat. Hot boning can cause meat to appear darker in colour compared to cold-boned meat (Warriss, 2010). At room temperature, the yeast, *Pseudomonas spp Achromobacter liquefaciens, Flavobacterium rhenanus* caused rapid colour changes. At 4 ± 1˚C' the aerobic bacteria cause active pigment changed from red to brown and finally to purple (Robach and Costilow, 1961). Thus, Sample G (purple) was ranked as very undesirable fresh beef.

According to Kreikemeier *et al.* (1998); Abril *et al.* (2001) and Honkavaara *et al.* (2003), an increase in glycolysis results from excessive excitement, starving and stress caused by ambient temperature, which in turn leads to high post-mortem pH values and consequently meat colour is influenced. The ultimate pH of meat affects everything from its colour, tenderness and eating quality. At a lower pH (below 5.3) meat will be pale and soft due to denaturation of myoglobin resulting in less desirable appearance and may be associated with other undesirable characteristics in fresh meat. Similar situation was witnessed in the study with respect to sample F (pale white) ranked very undesirable.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study has revealed that, *Salmonella* and *Escherichia coli* were present in the beef, and related contact surfaces at retail outlets in the Techiman Municipality. Therefore the beef supplied to the public are contaminated with *Salmonella* and *Escherichia coli*. However the presence of the microbial isolates stated above is worrying due to their ability to cause diseases. Unhygienic handling by butchers and retailers, transportation, storage, sanitary conditions at the various retail outlets, and environmental conditions may be the most probable sources of contamination.

Meat quality is the sum of three principal components: nutritional value, safety and consumer acceptability. During food preparation and display for the market it is necessary to ensure that conditions under which the meat is exposed would preserve safety and beauty. Consumers accepted beef with bright red cherry colours and rejected beef with colours that does not appeared attractive to them. The colour of beef and beef products is an important aspect of consumer acceptability and it influences the purchase decision by consumers as portrayed by this study. Meat purchasing decisions are influenced by colour more than any other quality factor because consumers use colouration as an indicator of freshness and wholesomeness.

6.2 Recommendation

Hygienic practices should be strictly adhered to, before, during and after each day's work by butchers in the Municipality beef retail markets and should be a shared responsibility among retailers, consumers and stakeholders to ensure that beef supplied to the public are safe for consumption.

Tools and equipment should be sterilized in between carcasses and cuts of beef and tables should be made with formica to ensure easy cleaning to prevent microbes inhabiting in crevices.

Butchers / retailers in the beef industry in the Municipality should be made to know consumers desire and perceptions on beef colour and to adequately equip and implement necessary knowledge and skills to deal with beef colour and its stability as well as other quality issues pertinent to consumers.

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APPENDIX

Appenix I: Media and reagent preparation

Peptone (Buffered) water (PW):

Formula	gram/litre
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5
pH	7.2 ± 0.2

25.5g of peptone was added to 1 litre of distilled water. It was warmed to dissolve and distributed into final containers and sterilise by autoclaving at 121 °C for 15 minutes (Bolton and Robertson, 1982).

Rappaport-Vassiliadis (Rv) Enrichment Broth

Formula (Classical)	gram/litre
Soya peptone	5.0
Sodium chloride	8.0
Potassium dihydrogen phosphate	1.6
Magnesium chloride $6H_2O$	40.0
Malachite green	0.04
pH	5.2 ± 0.2

Add 30g to 1 litre of distilled water. Heat gently until dissolved completely. Dispense 10ml volumes into screw-capped bottles or tubes and sterilise by autoclaving at 115°C for 15 minutes (Rappaport *et al.*, 1956)

Selenite Cystine (SC) Broth:

Formula	**gram/litre**
Tryptone	5.0
Lactose	4.0
Disodium phosphate	10.0
L-Cystine	0.01
pH	7.0 + 0.2

19g of Selenite Cystine Broth Base was dissolve in 1 litre of distilled water. Warmed to dissolve and 10ml dispensed into screw-capped bottles and sterilise by water bath for 15 minutes (Fricker 1987).

Xylose Lysine Deoxycholate Agar (XLD):

Formula	**gram/litre**
Yeast extract	3.0
L-Lysine HCl	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0

Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5
pH	7.4 ± 0.2

53g of XLD Agar was suspended in 1 litre of distilled water. Heated with frequent agitation until the medium started about to boil. The medium was poured into plates as soon as it cools(60°C) and allowed to solidify (Wissman *et al.*, 1975).

Brilliant Green Agar (BGA)

Formula	**gram/litre**
`Lab-Lemco' powder	5.0
Peptone	10.0
Yeast extract	3.0
Disodium hydrogen phosphate	1.0
Sodium dihydrogen phosphate	0.6
Lactose	10.0
Sucrose	10.0
Phenol red	0.09
Brilliant green	0.0047
Agar	12.0
pH	6.9 ± 0.2

52g was added in 1litre of distilled water and heated gently with agitation until when the medium was about to boil to dissolve the medium completely. The medium was allowed to cool, then poured into plates and allowed to solidify (Downes and Ito, 2001).

Nutrient Agar (NA):

Formula	**gram/litre**
`Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0
pH	7.4 + 0.2

28g was mixed in 1 litre of distilled water and heated until it was about to boil to dissolve completely and sterilised by autoclaving at 121°C for 15 minutes. After which it was poured into plates and allowed to cool (Bolton and Robertson, 1982).

Triple sugar iron (TSI):

Formula	**gram/litre**
`Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0

Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	q.s
Agar	12.0
pH	7.4 ± 0.2

65g of TSI agar was mixed in 1 litre of distilled water and heated until it dissolve completely. It was sterilised by autoclaving at 121°C for 15 minutes, transferred into tubes and allowed to cool (MacFaddin, 2000)

Lysine Iron Agar (LIA):

Formula	**gram/litre**
Bacteriological peptone	5.0
Yeast extract	3.0
Glucose	1.0
L-lysine	10.0
Ferric ammonium citrate	0.5
Sodium thiosulphate	0.04
Bromocresol purple	0.02
Agar	14.5
pH	6.7 ± 0.2

34g was suspended in 1 litre of distilled water. Heated until about to boil to dissolve completely. Dispensed into tubes and sterilised by autoclaving at 121°C for 15 minutes and allowed to cool (Finegold and Martin, 1982).

Simmon Citrate agar (CM0155)

Formula	**gram/litre**
Magnesium Sulphate	0 .2
Ammonium dihydrogen sulphate	0.2
Sodium ammonium phosphate	0.8
Sodium Citrate tribasic	2.0
Sodium chloride	2.0
Bromomethymol blue	0.08
Agar	15.0
pH	7.0 ± 0.2

23g was suspended in 1 litre of distilled water. It was heated to dissolve completely. Dispensed into tubes and sterilised by autoclaving at 121°C for 15 minutes and allowed to cool (APHA, 2011)

Levine Eosin Methylene Blue (LEMB) Agar:

Formula	**gram/litre**
Peptone	10.0
Lactose	10.0
Di-potassium hydrogen phosphate	2.0
Eosin	0.4

Methylene blue	0.06
Agar	15.0
pH	6.8 ± 0.2

37.5g was suspended in 1 litre of distilled water. Heated until about to boil to dissolve completely. Sterilised by autoclaving at 121 °C for 15 minutes and allowed to cool to 60°C.

Shake the medium to oxidize the methylene blue and suspend the precipitate which is an essential part of the medium (APHA, 2011)

MacConkey Agar:

Formula	**gram/litre**
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	0.075
Agar	12.0
pH	7.4 ± 0.2

52 g was suspended in 1 litre of distilled water. It was heated until it dissolved completely. Sterilised by autoclaving at 121°C for 15 minutes. Allowed surface of the gel to solidify for inoculation of the bacteria (Anderson, 2013)

Appendix II Pairwise comparisons of estimated marginal means based on the original scale of dependent variable of retail shops (P>0.05)

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference	
						Lower	Upper
Abanim	Ahenfie market	.06	.146	1	.668	-.22	.35
	Anyinabrem	.69[a]	.128	1	.000	.44	.94
	Brigade	.63[a]	.138	1	.000	.35	.90
	Dwomor market	.44[a]	.155	1	.005	.13	.74
	Hansua	.63[a]	.138	1	.000	.35	.90
	Kenten	.75[a]	.115	1	.000	.52	.98
	Main market	.75[a]	.115	1	.000	.52	.98
	Nana Abena market	.50[a]	.151	1	.001	.20	.80
	Sansama junction	-.12	.115	1	.276	-.35	.10
	Site	.63[a]	.138	1	.000	.35	.90
	Slaughter house	.69[a]	.128	1	.000	.44	.94
	Takofiano market	.19	.155	1	.228	-.12	.49

	Zongo Market	-.06	.128	1	.625	-.31	.19
	Zongo-Tamale Station	.00	.138	1	1.000	-.27	.27
Ahenfie market	Abanim	-.06	.146	1	.668	-.35	.22
	Anyinabrem	.63[a]	.136	1	.000	.36	.89
	Brigade	.56[a]	.146	1	.000	.28	.85
	Dwomor market	.37[a]	.162	1	.021	.06	.69
	Hansua	.56[a]	.146	1	.000	.28	.85
	Kenten	.69[a]	.124	1	.000	.44	.93
	Main market	.69[a]	.124	1	.000	.44	.93
	Nana Abena market	.44[a]	.159	1	.006	.13	.75
	Sansama junction	-.19	.124	1	.131	-.43	.06
	Site	.56[a]	.146	1	.000	.28	.85
	Slaughter house	.63[a]	.136	1	.000	.36	.89
	Takofiano market	.13	.162	1	.441	-.19	.44
	Zongo Market	-.13	.136	1	.359	-.39	.14
	Zongo-Tamale Station	-.06	.146	1	.668	-.35	.22
Anyinab	Abanim	-.69[a]	.128	1	.000	-.94	-.44

rem	Ahenfie market	-.63[a]	.136	1	.000	-.89	-.36
	Brigade	-.06	.128	1	.625	-.31	.19
	Dwomor market	-.25	.147	1	.088	-.54	.04
	Hansua	-.06	.128	1	.625	-.31	.19
	Kenten	.06	.102	1	.542	-.14	.26
	Main market	.06	.102	1	.542	-.14	.26
	Nana Abena market	-.19	.142	1	.188	-.47	.09
	Sansama junction	-.81[a]	.102	1	.000	-1.01	-.61
	Site	-.06	.128	1	.625	-.31	.19
	Slaughter house	.00	.117	1	1.000	-.23	.23
	Takofiano market	-.50[a]	.147	1	.001	-.79	-.21
	Zongo Market	-.75[a]	.117	1	.000	-.98	-.52
	Zongo-Tamale Station	-.69[a]	.128	1	.000	-.94	-.44
Brigade	Abanim	-.63[a]	.138	1	.000	-.90	-.35
	Ahenfie market	-.56[a]	.146	1	.000	-.85	-.28
	Anyinabrem	.06	.128	1	.625	-.19	.31
	Dwomor market	-.19	.155	1	.228	-.49	.12

	Hansua	.00	.138	1	1.000	-.27	.27
	Kenten	.12	.115	1	.276	-.10	.35
	Main market	.12	.115	1	.276	-.10	.35
	Nana Abena market	-.12	.151	1	.409	-.42	.17
	Sansama junction	-.75[a]	.115	1	.000	-.98	-.52
	Site	.00	.138	1	1.000	-.27	.27
	Slaughter house	.06	.128	1	.625	-.19	.31
	Takofiano market	-.44[a]	.155	1	.005	-.74	-.13
	Zongo Market	-.69[a]	.128	1	.000	-.94	-.44
	Zongo-Tamale Station	-.62[a]	.138	1	.000	-.90	-.35
Dwomor market	Abanim	-.44[a]	.155	1	.005	-.74	-.13
	Ahenfie market	-.37[a]	.162	1	.021	-.69	-.06
	Anyinabrem	.25	.147	1	.088	-.04	.54
	Brigade	.19	.155	1	.228	-.12	.49
	Hansua	.19	.155	1	.228	-.12	.49
	Kenten	.31[a]	.135	1	.021	.05	.58
	Main market	.31[a]	.135	1	.021	.05	.58
	Nana Abena market	.06	.168	1	.709	-.27	.39

	Sansama junction	-.56[a]	.135	1	.000	-.83	-.30
	Site	.19	.155	1	.228	-.12	.49
	Slaughter house	.25	.147	1	.088	-.04	.54
	Takofiano market	-.25	.171	1	.144	-.59	.09
	Zongo Market	-.50[a]	.147	1	.001	-.79	-.21
	Zongo-Tamale Station	-.44[a]	.155	1	.005	-.74	-.13
Hansua	Abanim	-.63[a]	.138	1	.000	-.90	-.35
	Ahenfie market	-.56[a]	.146	1	.000	-.85	-.28
	Anyinabrem	.06	.128	1	.625	-.19	.31
	Brigade	.00	.138	1	1.000	-.27	.27
	Dwomor market	-.19	.155	1	.228	-.49	.12
	Kenten	.12	.115	1	.276	-.10	.35
	Main market	.12	.115	1	.276	-.10	.35
	Nana Abena market	-.12	.151	1	.409	-.42	.17
	Sansama junction	-.75[a]	.115	1	.000	-.98	-.52
	Site	.00	.138	1	1.000	-.27	.27
	Slaughter house	.06	.128	1	.625	-.19	.31

	Takofiano market	-.44[a]	.155	1	.005	-.74	-.13
	Zongo Market	-.69[a]	.128	1	.000	-.94	-.44
	Zongo-Tamale Station	-.62[a]	.138	1	.000	-.90	-.35
Kenten	Abanim	-.75[a]	.115	1	.000	-.98	-.52
	Ahenfie market	-.69[a]	.124	1	.000	-.93	-.44
	Anyinabrem	-.06	.102	1	.542	-.26	.14
	Brigade	-.12	.115	1	.276	-.35	.10
	Dwomor market	-.31[a]	.135	1	.021	-.58	-.05
	Hansua	-.12	.115	1	.276	-.35	.10
	Main market	.00	.086	1	1.000	-.17	.17
	Nana Abena market	-.25	.131	1	.056	-.51	.01
	Sansama junction	-.87[a]	.086	1	.000	-1.04	-.71
	Site	-.12	.115	1	.276	-.35	.10
	Slaughter house	-.06	.102	1	.542	-.26	.14
	Takofiano market	-.56[a]	.135	1	.000	-.83	-.30
	Zongo Market	-.81[a]	.102	1	.000	-1.01	-.61

	Zongo-Tamale Station	-.75[a]	.115	1	.000	-.98	-.52
Main market	Abanim	-.75[a]	.115	1	.000	-.98	-.52
	Ahenfie market	-.69[a]	.124	1	.000	-.93	-.44
	Anyinabrem	-.06	.102	1	.542	-.26	.14
	Brigade	-.12	.115	1	.276	-.35	.10
	Dwomor market	-.31[a]	.135	1	.021	-.58	-.05
	Hansua	-.12	.115	1	.276	-.35	.10
	Kenten	.00	.086	1	1.000	-.17	.17
	Nana Abena market	-.25	.131	1	.056	-.51	.01
	Sansama junction	-.87[a]	.086	1	.000	-1.04	-.71
	Site	-.12	.115	1	.276	-.35	.10
	Slaughter house	-.06	.102	1	.542	-.26	.14
	Takofiano market	-.56[a]	.135	1	.000	-.83	-.30
	Zongo Market	-.81[a]	.102	1	.000	-1.01	-.61
	Zongo-Tamale Station	-.75[a]	.115	1	.000	-.98	-.52
Nana Abena market	Abanim	-.50[a]	.151	1	.001	-.80	-.20
	Ahenfie market	-.44[a]	.159	1	.006	-.75	-.13

	Anyinabrem	.19	.142	1	.188	-.09	.47
	Brigade	.12	.151	1	.409	-.17	.42
	Dwomor market	-.06	.168	1	.709	-.39	.27
	Hansua	.12	.151	1	.409	-.17	.42
	Kenten	.25	.131	1	.056	.00	.51
	Main market	.25	.131	1	.056	.00	.51
	Sansama junction	-.62[a]	.131	1	.000	-.88	-.37
	Site	.12	.151	1	.409	-.17	.42
	Slaughter house	.19	.142	1	.188	-.09	.47
	Takofiano market	-.31	.168	1	.062	-.64	.02
	Zongo Market	-.56[a]	.142	1	.000	-.84	-.28
	Zongo-Tamale Station	-.50[a]	.151	1	.001	-.80	-.20
Sansama junction	Abanim	.12	.115	1	.276	-.10	.35
	Ahenfie market	.19	.124	1	.131	-.06	.43
	Anyinabrem	.81[a]	.102	1	.000	.61	1.01
	Brigade	.75[a]	.115	1	.000	.52	.98
	Dwomor market	.56[a]	.135	1	.000	.30	.83
	Hansua	.75[a]	.115	1	.000	.52	.98

	Kenten	.87[a]	.086	1	.000	.71	1.04
	Main market	.87[a]	.086	1	.000	.71	1.04
	Nana Abena market	.62[a]	.131	1	.000	.37	.88
	Site	.75[a]	.115	1	.000	.52	.98
	Slaughter house	.81[a]	.102	1	.000	.61	1.01
	Takofiano market	.31[a]	.135	1	.021	.05	.58
	Zongo Market	.06	.102	1	.542	-.14	.26
	Zongo-Tamale Station	.12	.115	1	.276	-.10	.35
Site	Abanim	-.63[a]	.138	1	.000	-.90	-.35
	Ahenfie market	-.56[a]	.146	1	.000	-.85	-.28
	Anyinabrem	.06	.128	1	.625	-.19	.31
	Brigade	.00	.138	1	1.000	-.27	.27
	Dwomor market	-.19	.155	1	.228	-.49	.12
	Hansua	.00	.138	1	1.000	-.27	.27
	Kenten	.12	.115	1	.276	-.10	.35
	Main market	.12	.115	1	.276	-.10	.35
	Nana Abena market	-.12	.151	1	.409	-.42	.17
	Sansama junction	-.75[a]	.115	1	.000	-.98	-.52

	Slaughter house	.06	.128	1	.625	-.19	.31
	Takofiano market	-.44[a]	.155	1	.005	-.74	-.13
	Zongo Market	-.69[a]	.128	1	.000	-.94	-.44
	Zongo-Tamale Station	-.62[a]	.138	1	.000	-.90	-.35
Slaughter house	Abanim	-.69[a]	.128	1	.000	-.94	-.44
	Ahenfie market	-.63[a]	.136	1	.000	-.89	-.36
	Anyinabrem	.00	.117	1	1.000	-.23	.23
	Brigade	-.06	.128	1	.625	-.31	.19
	Dwomor market	-.25	.147	1	.088	-.54	.04
	Hansua	-.06	.128	1	.625	-.31	.19
	Kenten	.06	.102	1	.542	-.14	.26
	Main market	.06	.102	1	.542	-.14	.26
	Nana Abena market	-.19	.142	1	.188	-.47	.09
	Sansama junction	-.81[a]	.102	1	.000	-1.01	-.61
	Site	-.06	.128	1	.625	-.31	.19
	Takofiano market	-.50[a]	.147	1	.001	-.79	-.21
	Zongo Market	-.75[a]	.117	1	.000	-.98	-.52

	Zongo-Tamale Station	-.69[a]	.128	1	.000	-.94	-.44
Takofiano market	Abanim	-.19	.155	1	.228	-.49	.12
	Ahenfie market	-.13	.162	1	.441	-.44	.19
	Anyinabrem	.50[a]	.147	1	.001	.21	.79
	Brigade	.44[a]	.155	1	.005	.13	.74
	Dwomor market	.25	.171	1	.144	-.09	.59
	Hansua	.44[a]	.155	1	.005	.13	.74
	Kenten	.56[a]	.135	1	.000	.30	.83
	Main market	.56[a]	.135	1	.000	.30	.83
	Nana Abena market	.31	.168	1	.062	-.02	.64
	Sansama junction	-.31[a]	.135	1	.021	-.58	-.05
	Site	.44[a]	.155	1	.005	.13	.74
	Slaughter house	.50[a]	.147	1	.001	.21	.79
	Zongo Market	-.25	.147	1	.088	-.54	.04
	Zongo-Tamale Station	-.19	.155	1	.228	-.49	.12
Zongo Market	Abanim	.06	.128	1	.625	-.19	.31
	Ahenfie market	.13	.136	1	.359	-.14	.39

	Anyinabrem	.75[a]	.117	1	.000	.52	.98
	Brigade	.69[a]	.128	1	.000	.44	.94
	Dwomor market	.50[a]	.147	1	.001	.21	.79
	Hansua	.69[a]	.128	1	.000	.44	.94
	Kenten	.81[a]	.102	1	.000	.61	1.01
	Main market	.81[a]	.102	1	.000	.61	1.01
	Nana Abena market	.56[a]	.142	1	.000	.28	.84
	Sansama junction	-.06	.102	1	.542	-.26	.14
	Site	.69[a]	.128	1	.000	.44	.94
	Slaughter house	.75[a]	.117	1	.000	.52	.98
	Takofiano market	.25	.147	1	.088	-.04	.54
	Zongo-Tamale Station	.06	.128	1	.625	-.19	.31
Zongo-Tamale Station	Abanim	.00	.138	1	1.000	-.27	.27
	Ahenfie market	.06	.146	1	.668	-.22	.35
	Anyinabrem	.69[a]	.128	1	.000	.44	.94
	Brigade	.62[a]	.138	1	.000	.35	.90
	Dwomor market	.44[a]	.155	1	.005	.13	.74
	Hansua	.62[a]	.138	1	.000	.35	.90

	Kenten	.75[a]	.115	1	.000	.52	.98
	Main market	.75[a]	.115	1	.000	.52	.98
	Nana Abena market	.50[a]	.151	1	.001	.20	.80
	Sansama junction	-.12	.115	1	.276	-.35	.10
	Site	.62[a]	.138	1	.000	.35	.90
	Slaughter house	.69[a]	.128	1	.000	.44	.94
	Takofiano market	.19	.155	1	.228	-.12	.49
	Zongo Market	-.06	.128	1	.625	-.31	.19

Appendix III Pairwise comparisons of estimated marginal means based on the original scale of dependent variable retail shops (*Escherichia coli*) (P>0.05)

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference	
						Lower	Upper
Abanim	Ahenfie market	.38[a]	.150	1	.012	.08	.67
	Anyinabrem	.44[a]	.139	1	.002	.17	.71
	Brigade	.31[a]	.159	1	.049	.00	.62
	Dwomor market	.31[a]	.159	1	.049	.00	.62
	Hansua market	.50[a]	.125	1	.000	.26	.74
	Kenten market	.44[a]	.139	1	.002	.17	.71
	Main market	.38[a]	.150	1	.012	.08	.67
	NanaAbena market	.38[a]	.150	1	.012	.08	.67
	Sansama junction	-.13	.174	1	.472	-.47	.22
	Site	.31[a]	.159	1	.049	.00	.62
	Slaughter house	.44[a]	.139	1	.002	.17	.71

	Takofiannor market	.25	.165	1	.131	-.07	.57
	Zongo Market	.12	.174	1	.472	-.22	.47
	Zongo-Tamale Station	.25	.165	1	.131	-.07	.57
Ahenfie market	Abanim	-.38[a]	.150	1	.012	-.67	-.08
	Anyinabrem	.06	.102	1	.542	-.14	.26
	Brigade	-.06	.128	1	.625	-.31	.19
	Dwomor market	-.06	.128	1	.625	-.31	.19
	Hansua market	.12	.083	1	.131	-.04	.29
	Kenten market	.06	.102	1	.542	-.14	.26
	Main market	.00	.117	1	1.000	-.23	.23
	Nana Abena market	.00	.117	1	1.000	-.23	.23
	Sansama junction	-.50[a]	.147	1	.001	-.79	-.21
	Site	-.06	.128	1	.625	-.31	.19
	Slaughter house	.06	.102	1	.542	-.14	.26
	Takofiano market	-.13	.136	1	.359	-.39	.14
	Zongo Market	-.25	.147	1	.088	-.54	.04

	Zongo-Tamale Station	-.12	.136	1	.359	-.39	.14
Anyinabrem	Abanim	-.44[a]	.139	1	.002	-.71	-.17
	Ahenfie market	-.06	.102	1	.542	-.26	.14
	Brigade	-.13	.115	1	.276	-.35	.10
	Dwomor market	-.13	.115	1	.276	-.35	.10
	Hansua market	.06	.061	1	.302	-.06	.18
	Kenten market	.00	.086	1	1.000	-.17	.17
	Main market	-.06	.102	1	.542	-.26	.14
	Nana Abena market	-.06	.102	1	.542	-.26	.14
	Sansama junction	-.56[a]	.135	1	.000	-.83	-.30
	Site	-.13	.115	1	.276	-.35	.10
	Slaughter house	.00	.086	1	1.000	-.17	.17
	Takofiano market	-.19	.124	1	.131	-.43	.06
	Zongo Market	-.31[a]	.135	1	.021	-.58	-.05
	Zongo-Tamale Station	-.19	.124	1	.131	-.43	.06
Brigade	Abanim	-.31[a]	.159	1	.049	-.62	.00

	Ahenfie market	.06	.128	1	.625	-.19	.31
	Anyinabrem	.13	.115	1	.276	-.10	.35
	Dwomor market	.00	.138	1	1.000	-.27	.27
	Hansua market	.19	.098	1	.055	.00	.38
	Kenten market	.13	.115	1	.276	-.10	.35
	Main market	.06	.128	1	.625	-.19	.31
	Nana Abena market	.06	.128	1	.625	-.19	.31
	Sansama junction	-.44[a]	.155	1	.005	-.74	-.13
	Site	.00	.138	1	1.000	-.27	.27
	Slaughter house	.13	.115	1	.276	-.10	.35
	Takofiano market	-.06	.146	1	.668	-.35	.22
	Zongo Market	-.19	.155	1	.228	-.49	.12
	Zongo-Tamale Station	-.06	.146	1	.668	-.35	.22
Dwomor market	Abanim	-.31[a]	.159	1	.049	-.62	.00
	Ahenfie market	.06	.128	1	.625	-.19	.31
	Anyinabrem	.13	.115	1	.276	-.10	.35

	Zongo-Tamale Station	-.12	.136	1	.359	-.39	.14
Anyinabrem	Abanim	-.44[a]	.139	1	.002	-.71	-.17
	Ahenfie market	-.06	.102	1	.542	-.26	.14
	Brigade	-.13	.115	1	.276	-.35	.10
	Dwomor market	-.13	.115	1	.276	-.35	.10
	Hansua market	.06	.061	1	.302	-.06	.18
	Kenten market	.00	.086	1	1.000	-.17	.17
	Main market	-.06	.102	1	.542	-.26	.14
	Nana Abena market	-.06	.102	1	.542	-.26	.14
	Sansama junction	-.56[a]	.135	1	.000	-.83	-.30
	Site	-.13	.115	1	.276	-.35	.10
	Slaughter house	.00	.086	1	1.000	-.17	.17
	Takofiano market	-.19	.124	1	.131	-.43	.06
	Zongo Market	-.31[a]	.135	1	.021	-.58	-.05
	Zongo-Tamale Station	-.19	.124	1	.131	-.43	.06
Brigade	Abanim	-.31[a]	.159	1	.049	-.62	.00

	Zongo-Tamale Station	-.12	.136	1	.359	-.39	.14
Anyinabrem	Abanim	-.44[a]	.139	1	.002	-.71	-.17
	Ahenfie market	-.06	.102	1	.542	-.26	.14
	Brigade	-.13	.115	1	.276	-.35	.10
	Dwomor market	-.13	.115	1	.276	-.35	.10
	Hansua market	.06	.061	1	.302	-.06	.18
	Kenten market	.00	.086	1	1.000	-.17	.17
	Main market	-.06	.102	1	.542	-.26	.14
	Nana Abena market	-.06	.102	1	.542	-.26	.14
	Sansama junction	-.56[a]	.135	1	.000	-.83	-.30
	Site	-.13	.115	1	.276	-.35	.10
	Slaughter house	.00	.086	1	1.000	-.17	.17
	Takofiano market	-.19	.124	1	.131	-.43	.06
	Zongo Market	-.31[a]	.135	1	.021	-.58	-.05
	Zongo-Tamale Station	-.19	.124	1	.131	-.43	.06
Brigade	Abanim	-.31[a]	.159	1	.049	-.62	.00

	Ahenfie market	.06	.128	1	.625	-.19	.31
	Anyinabrem	.13	.115	1	.276	-.10	.35
	Dwomor market	.00	.138	1	1.000	-.27	.27
	Hansua market	.19	.098	1	.055	.00	.38
	Kenten market	.13	.115	1	.276	-.10	.35
	Main market	.06	.128	1	.625	-.19	.31
	Nana Abena market	.06	.128	1	.625	-.19	.31
	Sansama junction	-.44[a]	.155	1	.005	-.74	-.13
	Site	.00	.138	1	1.000	-.27	.27
	Slaughter house	.13	.115	1	.276	-.10	.35
	Takofiano market	-.06	.146	1	.668	-.35	.22
	Zongo Market	-.19	.155	1	.228	-.49	.12
	Zongo-Tamale Station	-.06	.146	1	.668	-.35	.22
Dwomor market	Abanim	-.31[a]	.159	1	.049	-.62	.00
	Ahenfie market	.06	.128	1	.625	-.19	.31
	Anyinabrem	.13	.115	1	.276	-.10	.35

	Brigade	.00	.138	1	1.000	-.27	.27
	Hansua market	.19	.098	1	.055	.00	.38
	Kenten market	.13	.115	1	.276	-.10	.35
	Main market	.06	.128	1	.625	-.19	.31
	Nana Abena market	.06	.128	1	.625	-.19	.31
	Sansama junction	-.44[a]	.155	1	.005	-.74	-.13
	Site	.00	.138	1	1.000	-.27	.27
	Slaughter house	.13	.115	1	.276	-.10	.35
	Takofiano market	-.06	.146	1	.668	-.35	.22
	Zongo Market	-.19	.155	1	.228	-.49	.12
	Zongo-Tamale Station	-.06	.146	1	.668	-.35	.22
Hansua market	Abanim	-.50[a]	.125	1	.000	-.74	-.26
	Ahenfie market	-.12	.083	1	.131	-.29	.04
	Anyinabrem	-.06	.061	1	.302	-.18	.06
	Brigade	-.19	.098	1	.055	-.38	.00
	Dwomor market	-.19	.098	1	.055	-.38	.00

	Kenten market	-.06	.061	1	.302	-.18	.06
	Main market	-.12	.083	1	.131	-.29	.04
	Nana Abena market	-.12	.083	1	.131	-.29	.04
	Sansama market junction	-.62[a]	.121	1	.000	-.86	-.39
	Site	-.19	.098	1	.055	-.38	.00
	Slaughter house	-.06	.061	1	.302	-.18	.06
	Takofiano market	-.25[a]	.108	1	.021	-.46	-.04
	Zongo Market	-.37[a]	.121	1	.002	-.61	-.14
	Zongo-Tamale Station	-.25[a]	.108	1	.021	-.46	-.04
Kenten market	Abanim	-.44[a]	.139	1	.002	-.71	-.17
	Ahenfie market	-.06	.102	1	.542	-.26	.14
	Anyinabrem	.00	.086	1	1.000	-.17	.17
	Brigade	-.13	.115	1	.276	-.35	.10
	Dwomor market	-.13	.115	1	.276	-.35	.10
	Hansua market	.06	.061	1	.302	-.06	.18
	Main market	-.06	.102	1	.542	-.26	.14

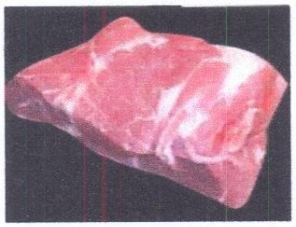
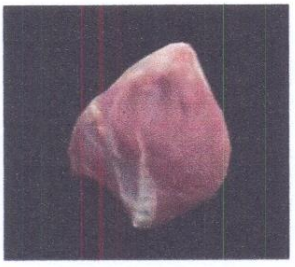
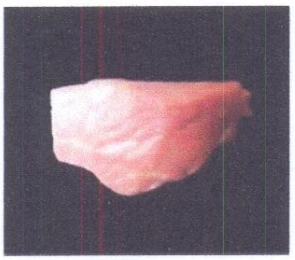
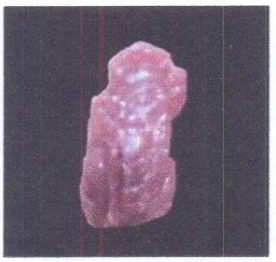
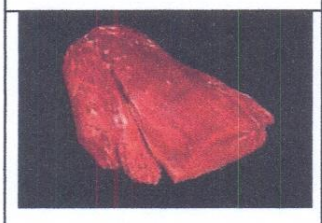
	Nana Abena market	-.06	.102	1	.542	-.26	.14
	Sansema junction	-.56[a]	.135	1	.000	-.83	-.30
	Site	-.13	.115	1	.276	-.35	.10
	Slaughter house	.00	.086	1	1.000	-.17	.17
	Takofiano market	-.19	.124	1	.131	-.43	.06
	Zongo Market	-.31[a]	.135	1	.021	-.58	-.05
	Zongo-Tamale Station	-.19	.124	1	.131	-.43	.06
Main market	Abanim	-.38[a]	.150	1	.012	-.67	-.08
	Ahenfie market	.00	.117	1	1.000	-.23	.23
	Anyinabrem	.06	.102	1	.542	-.14	.26
	Brigade	-.06	.128	1	.625	-.31	.19
	Dwomor market	-.06	.128	1	.625	-.31	.19
	Hansua market	.12	.083	1	.131	-.04	.29
	Kenten market	.06	.102	1	.542	-.14	.26
	Nana Abena market	.00	.117	1	1.000	-.23	.23
	Sansema junction	-.50[a]	.147	1	.001	-.79	-.21

	Site	-.06	.128	1	.625	-.31	.19
	Slaughter house	.06	.102	1	.542	-.14	.26
	Takofiano market	-.13	.136	1	.359	-.39	.14
	Zongo Market	-.25	.147	1	.088	-.54	.04
	Zongo-Tamale Station	-.12	.136	1	.359	-.39	.14
NanaAbena market	Abanim	-.38[a]	.150	1	.012	-.67	-.08
	Ahenfie market	.00	.117	1	1.000	-.23	.23
	Anyinabrem	.06	.102	1	.542	-.14	.26
	Brigade	-.06	.128	1	.625	-.31	.19
	Dwomor market	-.06	.128	1	.625	-.31	.19
	Hansua market	.12	.083	1	.131	-.04	.29
	Kenten market	.06	.102	1	.542	-.14	.26
	Main market	.00	.117	1	1.000	-.23	.23
	Sansema junction	-.50[a]	.147	1	.001	-.79	-.21
	Site	-.06	.128	1	.625	-.31	.19
	Slaughter house	.06	.102	1	.542	-.14	.26

	Takofiano market	-.13	.136	1	.359	-.39	.14
	Zongo Market	-.25	.147	1	.088	-.54	.04
	Zongo-Tamale Station	-.12	.136	1	.359	-.39	.14
Sansema junction	Abanim	.13	.174	1	.472	-.22	.47
	Ahenfie market	.50[a]	.147	1	.001	.21	.79
	Anyinabrem	.56[a]	.135	1	.000	.30	.83
	Brigade	.44[a]	.155	1	.005	.13	.74
	Dwomor market	.44[a]	.155	1	.005	.13	.74
	Hansua market	.62[a]	.121	1	.000	.39	.86
	Kenten market	.56[a]	.135	1	.000	.30	.83
	Main market	.50[a]	.147	1	.001	.21	.79
	NanaAbena market	.50[a]	.147	1	.001	.21	.79
	Site	.44[a]	.155	1	.005	.13	.74
	Slaughter house	.56[a]	.135	1	.000	.30	.83
	Takofiano market	.38[a]	.162	1	.021	.06	.69
	Zongo Market	.25	.171	1	.144	-.09	.59

	Zongo-Tamale Station	.38[a]	.162	1	.021	.06	.69
Site	Abanim	-.31[a]	.159	1	.049	-.62	.00
	Ahenfie market	.06	.128	1	.625	-.19	.31
	Anyinabrem	.13	.115	1	.276	-.10	.35
	Brigade	.00	.138	1	1.000	-.27	.27
	Dwomor market	.00	.138	1	1.000	-.27	.27
	Hansua market	.19	.098	1	.055	.00	.38
	Kenten market	.13	.115	1	.276	-.10	.35
	Main market	.06	.128	1	.625	-.19	.31
	Nana Abena market	.06	.128	1	.625	-.19	.31
	Sansema junction	-.44[a]	.155	1	.005	-.74	-.13
	Slaughter house	.13	.115	1	.276	-.10	.35
	Takofiano market	-.06	.146	1	.668	-.35	.22
	Zongo Market	-.19	.155	1	.228	-.49	.12
	Zongo-Tamale Station	-.06	.146	1	.668	-.35	.22
Slaughte	Abanim	-.44[a]	.139	1	.002	-.71	-.17

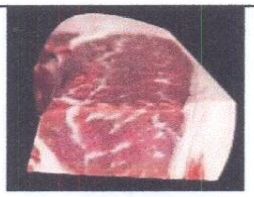
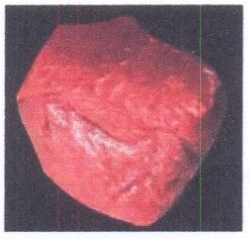
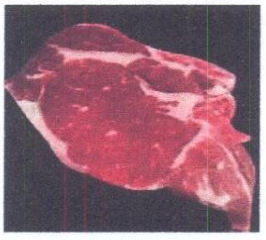
	NanaAbena market	.12	.136	1	.359	-.14	.39
	Sansema junction	-.38[a]	.162	1	.021	-.69	-.06
	Site	.06	.146	1	.668	-.22	.35
	Slaughter house	.19	.124	1	.131	-.06	.43
	Takofiano market	.00	.153	1	1.000	-.30	.30
	Zongo Market	-.13	.162	1	.441	-.44	.19

	**D -** Magenta								
	**E –** Crimson								
	**F -** Pale white								
	**G -** Purple magenta								
	**H –** Red								

Appendix IV

Subjective assessment of fresh beef colour Correspondent's Expectation when

buying mea/reason(s) for choice.....

Meat Samples (Colour)	Name/Colour Description	Extremely Desirable (1)	Very Desirable (2)	Moderately Desirable (3)	Slightly Desirable (4)	Slightly Undesirable (5)	Moderately Undesirable (6)	Very Undesirable (7)	Extremely undesirable (8)
	**A** – scarlet								
	**B** - Scarlet red								
	**C** - Bright cherry red								

Appendix V: Percentages of consumer preference of fresh beef colour

Sample/Pref.	1	2	3	4	5	6	7	8	AV
A	7.9	17.9	37.5	27.1	3.3	2.5	2.9	0.8	3.23
B	1.3	16.3	33.3	31.3	9.2	7.5	0.8	0.4	3.55
C	84.2	9.6	2.5	2.1	0.8	0.8	0	0	1.28
D	4.2	52.5	18.8	18.8	3.3	1.7	0.4	0.4	2.74
E	1.3	1.7	2.1	10	43.8	31	8.3	2.1	5.36
F	0	0.4	1.3	0.4	7.1	13	43.3	35	6.99
G	0	0.8	0.8	0.8	2.9	8.3	30	56	7.3
H	0.4	1.3	4.2	12.1	28.3	35	12.9	5.4	5.51

Appendix VI Means of consumer preferences of fresh beef colour at various retail markets

Location/Sample	A	B	C	D	E	F	G
Abanim	3.75	3.5	1.06	2.625	5.56	6.5	7.81
Ahenfie market	3.375	3.75	1.06	2.312	5.44	6.4	7.38
Anyinabrem	3	4.06	1.25	2.812	0.81	7	7.44
Brigade	3.188	3.31	1.31	2.688	5.63	6.6	7.06
Dwomor	2.562	3.38	1.31	2.688	5.38	7.3	7.47
Hansua	2.938	3.38	1	3.062	5.56	7.1	6.94
Kenten	3.625	3.69	2.06	2.812	4.44	6.6	6.56
Main market	3.625	3.06	1.06	2.75	5.19	6.9	7.5
Nana Abena Market	2.938	3.25	1.25	2.875	5.5	7.4	6.94
Sansama junction	3.437	2.88	1.69	3	5.25	6.9	7.13
Site	3.375	3.69	1.38	1.937	5.38	7.6	7.31
Slaughter house	3.125	4	1.25	2.75	5.31	7.1	7.06
Takofiano market	3.812	3.81	1.19	3.25	5.06	6.9	6.81
Zongo market	3.25	3.81	1	2.125	5.44	7.6	7.13
Zongo-Tamale station	2.688	3.63	1.38	3	5.44	6.9	7.63