KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI

DEPARTMENT OF ENVIRONMENTAL SCIENCE INSTITUTE

OF DISTANCE LEARNING

ASSESSMENT OF MICROBIAL QUALITY AND HEAVY METAL LEVELS OF RAW CATTLE HIDE AND MEAT SOLD AT RETAIL OUTLETS IN TARKWA, WESTERN REGION, GHANA

A Thesis Submitted to the Department of Environmental Science, Kwame Nkrumah University of Science and Technology, Kumasi, in Partial Fulfillment of the Award of Master of Science in Environmental Science

BY

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DECLARATION

I hereby declare that except for works of other authors which served as sources of references and information and which have been duly acknowledged, the work herein submitted as a thesis for the MSc. Environmental Science Degree, is the result of my own effort under supervision, and has not been presented nor is being concurrently submitted for any other degree elsewhere. I am thus responsible for the views expressed and the factual exactness of its contents.

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DEDICATION

This Thesis is dedicated to my Parents and In-laws who upheld and motivated me to take up this study. Your prayer and contribution is much appreciated.

To my wife Elizabeth Taylor, whose encouragement and support has seen me this far. I will forever be grateful.

To my siblings, your moral support kept me going in those boisterous times of my study.

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ABBREVIATIONS / ACRONYMS

AHVLA-Animal Health and Veterinary Laboratories Agency

AOAC-Association of official Analytical Chemists

ATSDR-Agency for Toxic Substances and Disease Registry

CDC-Centre for Disease and Control Prevention

CFU-Colony Forming Unit

CIA-Central Intelligence Agency

ECR-Europeans Commission's Regulation

EPA-Environmental Protecting Agency

FAO-Food and Agriculture Organization

FBD-Foodborne Diseases

GAEC-Ghana Atomic Energy Commission

GHP-Good Hygiene Practices

GSB-Ghana Standard Board

HACCPS-Hazard Analyses and Critical Control Point Scheme.

ICMS-International Commission on microbiological Specification

KIA-Kligers Iron Agar

MHMT-Municipal Health Management Team

MoFA-Ministry of Food and Agriculture

MPL’s-Maximum Permissible Limits

SPSS-Statistical Package for Social Science

TCC-Total Coliform Count

TSC-Total Staphylococcal Count

TVC-Total Viable Count

USDA-United States Department of Agriculture

VSD-Veterinary Service Directorate

WHO-World Health Organization

ABSTRACT

The present study seeks to assess the microbial quality of raw beef as well as the concentration of heavy metals in cattle hide at retail outlets in the Tarkwa Municipality. A total of 384 replicated swab samples were taken from beef, knives, wooden boards, weighing scales, shop floor as well as workers hands. Also 24 replicated freshly singed and unsinged cattle hides were aseptically collected. Sampling was done at selected retail outlets in Central Market (Layout) and Karikwanaano Markets.

Averagely, 2.55±0.27 (log_{10} cfu/cm²), 2.06±0.22 (log_{10} cfu/cm²), and 1.57±0.17 (log_{10} cfu/cm²) of total viable count (TVC), total coliform count (TCC) and total Staphylococcal Counts (TSC) were recorded respectively on all swab samples from retail outlets. There were significant microbial growth differences across the various retail sale environments. Microbial loads in the fresh swab sample (tvc = 1.36±0.21 (log_{10} cfu/cm²), tcc = 1.10±0.16 (log_{10} cfu/cm²), tsc = 0.87±0.13 (log_{10} cfu/cm²), were significantly lower than delayed swab samples (tvc = 3.74±0.37 (log_{10} cfu/cm²), tcc = 3.02±0.30 (log_{10} cfu/cm²), tsc = 2.28±0.24 (log_{10} cfu/cm²). Comparatively, a significant microbial growth ($p < 0.05$) was observed on the beef to the environmental equipment. The average pH reading in this study was slightly acidic (6.88±0.78), then peaking acidity in the afternoon. Foodborne pathogens isolated from beef, its processing equipments and the surrounding environment included *Staphylococcus spp.*, *Salmonella*, *Streptococcus spp.*, *Escherichia coli. Enterobacter spp.* and *Klebsiella spp.*

12 cattle hides were singed-treated with scrap tyres (T) while the remaining 12 were firewood processed. The control 24 hides were taken from the un-singed carcasses before the singeing took place. Samples of carcasses hides were analyzed for the concentrations of Fe, Pb, Cu and Zn. The average concentration of all heavy metal contents in hides recorded were lower than the maximum permissible limit except for Lead (Pb). However statistical significance difference between permissible limit (50mg/kg) and observed (23.44±5.70 mg/kg) was recorded only for iron (Fe).The type of processing method (unsinged, firewood-singed and tyre-singed) had a significant effect ($p < 0.05$) on the levels of heavy metal content recorded in hide. Hide processed with scrap tyre recorded the highest level of heavy metal concentration to fire wood-singed. There was no significance difference ($p > 0.05$) on the level of heavy metal content of hide among the two retail markets.

The microbial load of raw beef from retail outlet in Tarkwa Municipality is high which insinuates its possible role in spoilage in foodborne illness. Therefore there is the need for improvement in the standard of selling meat in Tarkwa Municipality. The hides treated with scrap tyres were unsafe for human consumption. Therefore there should be the enforcement of stringent laws in Ghana to stop local butchers from using scrap tyres as singeing material.

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CHAPTER ONE

INTRODUCTION

1.1 General Background

Meat has long been known for its nutritive composition, which explains why it is consumed by many people worldwide. The basic constituents of protein profile of meat consist of amino acids that have been described as excellent due to the presence of all essential amino acids required for the body. Meat is a major source of nutrient in our meals. However, it can also serve as a rich medium of growth for harmful microorganisms. The United Nations Environmental Program Report (2014), agreed on new standards to protect the health of consumers worldwide. It reported that, food can become contaminated by heavy metals, fungal toxins or bacteria and viruses.

WHO (1997) reported that, meat infected with microorganisms is the cause of many foodborne diseases. The animals (Animal Source Food) themselves or from outside may be the source of these pathogenic microorganisms. Contamination of meat by pathogenic microorganisms is as a result of the surroundings where these animals are kept as well as the way they are processed after slaughtering (Adeyemo, 2002).

Effective sanitation has a remarkable impact on the profit of meat processors and retailers by reducing spoilage and providing a longer shelf life. Proper sanitation also helps maintain meat colour, which leads to more sales and reduced labour cost (Adentunde *et al.*, 2011 and Obeng *et al.*, 2013). They further reported that, good sanitation will provide a healthy, clean environment, which upgrades the image and reputation of the store (sales point). While food borne diseases remain an important environment and public health problem worldwide, one of the most significant food safety hazards is associated with food from animals (AduGyamfi *et* *al.*, 2012; Kivi *et al*, 2007; Maripandi and Al-Salamah, 2010). A study by Fratamico *et al.* (2005) stated that food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs.

The WHO has described Food Borne Disease (FBD's) as illness of an infections or toxic nature caused by, or thought to have been caused by the consumption of food and water. It estimates up to a third of people in developed countries are affected by FDB's (WHO, 2009). Food and drugs Authority (FDA) in 2010 reported that, total number of out-patients reported with food borne diseases in Ghana in 2009 was about 20,000 per year, with an annual death rate estimate at 6,500 and total cost to the economy at US 69 million. According to the Ghana Health Service (GHS) the total number of sick persons associated with food-borne illnesses for the period of 2011 in Ghana was 2.2 million, whiles the associated losses for 2010, based on the hypothesis that food borne illness were diarrhoea diseases was GH¢ 29.4 million (approximately US$ 13.01million at a rate of 1$ to GH¢ 2.26 (Ghana Stock Exchange, January 2014). This figure came from health care costs to the Government, individuals and the loss of about 4.6 million working days (Ghana FAO/WB CP, 2012). Food borne diseases are caused by the consumption of foods exposed to hazards that may be biological or pathogenic (eg. Parasites, bacteria, viruses), chemical (eg. Toxins and heavy metals), and other physical (eg, bone chips, and glass fragments) (Melngaile *et al.*, 2014). Obeng *et al.*, (2013) and Khalafalla *et al.*, 2011 reported in their study that, although there could be the presence of contaminant on meats, it does not necessarily make the meat unwholesome. However the presence of the microbial isolates such as *Streptococcus spp.*, S*taphylococcus spp*., *Salmonella*, and *Escherichia coli* on meat sold in retail outlets is worrying due to their ability to cause diseases. Improper or unhygienic handling by butchers and retailers, processing, transportation, storage, sanitary conditions at various retail outlets, and environmental conditions may be the most probable sources of contamination. 62% of all human pathogens are zoonotic as reported by Mukhopadhyay *et al.* (2009), which more or less agrees with the World Organisation for Animal Health Report (2012) that states 75% of all emerging human diseases originate from animal reservoirs. Consequently, Animal Sourced Foods (ASF) have been found guilty for the majority of FBD's (Clarence *et al*., 2009), and incidences increase with increasing access to such foods especially without adequate hygiene, inspection for safety or satisfactory heating to kill pathogen (Kra *et al*., 2011).

Rapid urbanization and swift way of living, as a result of increased demand for ready to cook and ready to eat meat products have substantially changed the food habits of most Ghanaians as noted in Table 1.3 and its index of production of meat in Table 1.4 (MoFA, SRID, 2011). Some of the factors consumers consider in their choice of meat product are the quality and type, value for money, freshness and health aspects of meat food products (Selvan *et al*., 2007). There is a steady shift to Animal Source Food (ASF) across developing countries (DC's) (Popkin, 2003). This assertion reflects the gradual increase in animal meat production in Ghana, (Table 1.1) and therefore their production index, (Table 1.2) in response to an almost exclusive domestic demand (Ghana; MoFa: SRID, 2011).

Table 1.1 Livestock Population (1998-2009).

Types of livestock	Livestock Population ('000')											
	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Cattle	1,273	1,288	1,302	1,315	1,330	1,344	1,359	1,373	1,392	1,407	1,422	1,438
Sheep	2,576	2,658	2,743	2,771	2,922	3,015	3,112	3,211	3,314	3,420	3,529	3,642
Goats	2,792	2,931	3,077	3,199	3,230	3,560	3,925	3,923	3,997	4,196	4,405	4,625
Pigs	339	332	324	312	310	303	297	290	477	491	506	521
Poultry	17,302	18,810	20,472	22,032	24,251	26,395	28,727	28,386	34,030	37,038	39,816	43,320
TOTAL	24,282	26,019	27,918	29,629	32,043	34,617	37,420	37,183	43,210	46,552	49,678	53,546

Source: Statistics Research and Information Directorate (SRID) MoFA Accra.

Table 1.2 Index of Livestock Population (1999-2009).

Types of livestock	Index of Livestock Population (1997 = 100)										
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Cattle	102	103	104	106	107	108	109	110	112	113	114
Sheep	106	110	111	117	121	125	129	133	137	141	146
Goats	110	116	120	121	134	148	148	150	158	166	174
Pigs	96	93	90	89	87	86	84	137	141	146	150
Poultry	118	129	139	153	166	181	179	214	233	251	273
TOTAL	532	551	564	586	615	648	649	744	781	817	857

Source: Statistics Research and Information Directorate (SRID) MoFA Accra.

1.2 Raw Meat

Meat is considered as the most important source of proteins consumed by humans. In the same vein, meat is the most perishable of all staple foods since it contains sufficient nutrients needed to support the growth of microorganisms (Huda *et al*., 2010). For highly perishable foodstuffs such as fresh red meat, the threat of food poisoning is particularly high (Nel *et al*., 2004; Yousuf *et al*., 2008). Thus, if food is not immediately utilized or preserved after harvesting, it spoils. Adzitey *et al*. (2011), reported that, despite the roles cattle rearing play in the livelihood of most Ghanaians and the contribution to the daily protein intake, beef is a source of foodborne illnesses, especially under the condition in which animals are handled, slaughtered and transported as well as sold on Ghanaian markets. Prescott *et al.* (2002), also showed that, food items especially meat, were not only of high nutritional value to those who consume them but often are ideal culture media for microbial growth.

Mukhopadhyay *et al.,* (2009) also reported that fresh raw meat like beef have been implicated for a number of meat borne infections and intoxications in several countries. This is because both pathogenic and non-pathogenic organisms live in the gastro-intestinal tract of cattle which can be transferred onto the meat under faulty and poor processing conditions.

Contaminated raw meat is one of the main sources of food-borne illness (Bhandare *et al.,* 2007; Podpecan *et al*; 2007). Recent increase in the consumption of meat and its products arises from reasons including high protein contents, minerals, vitamins, lipids and savoury sensation. At the 18th session of Food and Agriculture Organisation (FAO) and WHO of the Codex Coordinating Committee for Africa, held in Accra, WHO reported that the disability adjusted life years lost to food and water borne diarrhoea in Africa region was 4.1 per 100 globally as compared to 5.7-7.1 per 100 in Africa (CODEX/FAO/WHO/WTO, 2009).

Table 1.3 Meat Productions in Ghana (1999-2009).

Types of livestock	Domestic Meat Production (Metric Tons)										
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Cattle	18,029	18,570	19,053	18,288	18,486	18,686	18,874	19,140	19,346	19,553	19,768
Sheep	11,940	12,298	12,780	13,149	13,568	14,004	14,450	14,913	15,390	15,881	16,389
Goats	11,216	11,552	12,037	12,597	13,884	15,308	15,300	15,588	16,364	17,180	18,038
Pigs	11,173	10,056	9,653	10,416	10,181	9,979	9,744	16,027	16,498	17,002	17,512
Poultry	14,534	13,807	14,580	19,401	21,116	22,982	22,709	27,224	29,630	31,853	34,656
TOTAL	66,892	66,283	68,103	73,851	77,235	80,959	81,077	92,892	97,228	101,469	106,363

Source: Statistics Research and Information Directorate (SRID) MoFA Accra

Table 1.4 Index of Meat Production (1999-2009).

Types of livestock	Index of Meat Production (1997 = 100)											
	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Cattle	101	105	108	111	107	108	109	110	112	113	114	115
Sheep	103	110	113	117	121	125	129	133	137	141	146	151
Goats	105	114	117	122	128	141	155	155	158	166	174	183
Pigs	98	98	89	85	92	90	88	86	141	145	150	154
Poultry	114	131	124	131	175	190	207	205	245	267	287	312
TOTAL	521	558	551	566	623	654	688	689	793	832	871	915

Source: Statistics Research and Information Directorate (SRID) MoFA Accra.

1.3: Quality of Meat Sold in Ghana

Ghana is a wet Sub-Saharan African country where commercial bovine cattle breeding are almost absent. So the country imports livestock up to 47% of its needs from Mali, Burkina Faso as well as processed beef from European countries (Table 1.5). Although beef is popular in Ghana, it accounts for only 39% of the meat produced (Table 1.3) and consumed (Table 1.4) (MoFA-Agriculture in Ghana; Facts and Figures, 2011).

In Ghana, fresh meats are sold every day at a retail level to the public on open markets particularly for low-income people who cannot afford a refrigerator for preservation. Since the animal carcasses are not stored in chill rooms after slaughtering, they must be sold within the same day. The perception of most Ghanaian meat consumers is that the internal tissues of healthy slaughtered animals are free of bacteria at the time of slaughtering. However, when the meat gets to the retail market, it may contain varying numbers and types of microorganisms (Nkegbe *et al*., 2013).

Although the Environmental Health Department as well as the Veterinary Services Department of the various MMDA's in Ghana are mandated to ensure safe, healthy and hygienic carcasses, foods such as fish or fresh meat is usually kept exposed while awaiting buyers or carried in sieves or slabs with it accompanying sounds to attract buyers. These eventually make it naturally vulnerable to contamination with different types of microorganisms. Therefore, improper handling and improper hygiene may lead to the contamination of fresh meats and eventually affect the health of the consumers (Koussemon *et al.,* 2008)

Although people were used to well cooked food and ate at home most of the time, the trend has changed of late. Many people eat out, buying ready to eat foods sold by the street sides that are most often undercooked. Dyckman and Lansburgh (2002) reported that, one of the most important food safety hazards is associated with undercooked meat and poultry. Street vending of foods is a common characteristic of countries with high unemployment rates, low salaries and poor social security programme.

In West Africa, especially in Ghana and Cote d'Ivoire, street-fast-processed and vended foods such as chicken and beef meat are not always well cooked and are eaten without further processing or cooking (Koffi-Nevry *et al.,* 2011). Hence, contaminated foods from fresh red meat infected with microorganisms, can lead to consumer health problems.

Ologhobo *et al*. (2010) reported that microbial counts of beef and chicken (street sides roasted meat) were at levels that pose health problems to consumers. Beef 'Kyinkyinga'; a Ghanaian local meat delicacy had earlier on been reported by Mensah *et al.* (2002). Feglo and Sakyi, 2012 and Ologhobo *et al.* (2010), to have contained pathogens such as *Staphylococcus spp.*, *Salmonella,* and entero-pathogenic *Escherichia coli*.

There are relatively few surveys and a lack of information on the bacteriological status of beef carcases offered for retail sale and the level of hygiene of the slaughter houses in Ghana.

It is therefore important to investigate some bacteria such as *E. coli* and *S. aureus* since they serve as indicators of food contaminants due to excessive human handling (Clarence *et al.*, 2009). *Pseudomonas*, a proteolysis contaminating food bacterium may be used to predict the level of spoilage of the beef carcasses while several foodborne outbreaks can be linked to *Salmonella* in meat and poultry (Dechet *et al.*, 2006; Aftab *et al.*, 2012; Thomas *et al.*, 2006). Typhoid fever continues to be a major environmental and public health concern in many countries such as Ghana.

1.4 Heavy Metals

Singeing in an open fire is the major process by which hair on the skin of slaughtered cattle is removed in most African countries including Ghana (Obiri-Danso *et al.*, 2008). This is done because singeing evokes flavours in the meat which are tolerable to the consumer and also preserves the carcass hide for consumption. Singeing is the process of burning the fur, hair or feathers superficially or slightly in order to expose the carcass of an animal (Okiel *et al.*, 2009). In Ghana, firewood as fuel is mainly used for singeing, but the relative dearth of firewood in current times has resulted in local butchers using scrap vehicular tyres in place of firewood (Obiri-Danso *et al.*, 2008). The contents of vehicular tyres are reported to have poisonous substances which could cause health threats to humans. The use of scrap tyres, according to the local butchers is inexpensive and more efficient, as it produces more flames with less heat hence it is able to selectively burn off the fur from the animal without cracking the hide (Adam *et al.*, 2013; Obiri-Danso *et al.*, 2008)

USFA, (1999) reported that scrap tyres are used as fuel because of their high heating value. However the use of car tyres for singeing has been reported by many authors to have some poisonous heavy metals to the singed skin that could diffuse into the meat, eventually contaminating, to making it hazardous for human consumption (USFA, 1999; Okiel *et al.*, 2009; Costa, 2000; Jayasekara *et al.*, 1992; Essumang *et al*., 2007; Eremong *et al*., 2011; Akwetey *et al*, 2013)

Tyres Derived Fuel (TDF's) contains several heavy metals including lead (Pb), Zinc (Zn), Copper (Cu) that could be carcinogenic when exposed to consumers continuously over a long period as reported by the Agency for Toxic Substances and Disease Registration of the United States of America. Adam *et al*. (2013); Obiri-Danso *et al*. (2008); and Akwetey *et al*. (2013) all reported high levels of heavy metals residues in cattle and goat hides singed with scrap tyres.

Consumer awareness for foods that are microbiologically and toxically safe is increasing tremendously in developed countries, which is not the case observed in most developing Therefore there is the need to produce meat that is of better quality and disease-free, especially in most developing Africa countries.

Table 1.5 Imports of Livestock and Poultry Products in Metric Tons (2000-2009).

CATEGORY	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
BOVINE:										
BEEF	631.6	73.2	901.3	1,112.40	2,586.80	6,331.70	10,585.50	16,250.40	13,135.1	12,338.4
BUFFALO	237.9	81	162.2	249.6	1,169.20	2,257.10	4,717.30	8,109.00	7,366.8	4,454.5
SUB--TOTAL	869.5	154.2	1063.5	1362	3756	8588.8	15302.8	24359.4	20501.9	16792.9
POULTRY:										
CHICKEN	9,160.0	6,731.50	19,986.0	32,939.0	39,088.60	40,591.00	44,757.70	63,276.30	89,889.0	67,068.6
TURKEY	385.9	74.1	766.3	1,164.50	1,268.70	1,697.20	3,030.30	3,514.70	3,352.8	1,980.2
DUCK	2.1	2	0	4.1	0	0	6.1	0	16	30.8
SUB--TOTAL	9,548.0	6,807.60	20,752.3	34,107.6	40,357.3	42,288.2	47,794.1	66,791.0	93,257.8	69,079.6
OTHER:										
CHEVON	74.4	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MUTTON	237.4	478.2	1,285.0	2,122.30	2,053.40	3,640.80	4,839.20	6,887.10	5,961.3	6,153.1
PORK	358.1	1,166.10	7,737.5	9,882.30	7,756.40	10,286.8	13,290.5	10,551.5	5,487.4	3,150.2
PROCESSED MEAT	106.8	80.4	133.9	0.00	256.2	270.4	0.00	0.00	0.00	0.00
SUB--TOTAL	776.7	1724.7	9156.4	12004.6	10066	14198	18129.7	17438.6	11448.7	9303.3
MILK	96	1,548.50	865.5	349.40	203.30	1,555.10	1,044.20	2,659.90	2,718.9	11,406.4
GRAND TOTAL	11,290.2	10,235.0	31,837.7	47,823.5	54,382.60	66,630.10	82,270.8	111,248.9	127,927.3	106,582.2

Source: Statistics Research and Information Directorate (SRID) MoFA Accra.

1.5: Statement of Problem

Ghanaians' are noted for consuming beef, chevon, chicken as well as mutton during festive seasons such as Christmas, Easter, marriage ceremonies, Eid al Adha, Eid al Fitr etc, as a source of food. The animals are normally slaughtered in abattoirs and sometimes in backyards without observing stringent hygienic practices. More so, it is common practice to see people carrying carcasses just after dressing on their bare shoulders and hands. The animal carcasses are usually transported to the markets either in meat vans (normally not well maintained), motor cycles, taxis, or bicycles. Meats are sold in the open markets sometimes in sieves or without sieves, and on tables that are usually not maintained or cleaned after work. Meat sellers and butchers pay less attention to their personal hygiene and serve meats with dirty hands and clothing.

Carcass and meat quality defects such as pale soft exudative, dark firm dry meat, skin blemish, blood splash, bruising, cyanosis, two-toning, high microbial load, spoilage of meat, broken bones and death may occur from improper animal handling (Adzitey *et al*., 2011).

Adu- Gyamfi *et al*. (2012) found various levels and numbers of total bacteria counts, *Escherichia coli*, *Salmonella*, *Staphylococcus spp* and *S. aureus* on chicken sold in Accra. Iddrisu's work (2007) on milk, raw cheese and fried cheese in the Tamale Metropolis also indicated the presence of *Escherichia coli, Streptococcus spp*. and *Staphylococcus spp*. on the sample. Also, *Escherichia coli*, *Salmonella*, *Staphylococcus spp* and *streptococcus spp* were reported to contaminate beef sold in the Tamale Metropolis (Kutah, 2010)

Beef, chevon and mutton contamination by various microorganisms have also been reported in other countries by different authors. In Tamil Nadu; India, Bradeeba and Sivakumaar (2013) found that the mean total viable count was not significantly ($p>0.05$) different in beef, mutton and pork meat.

In the traditional customs and values of some tribes in Ghana, certain diseases were classified as sacred, ancestral punishment and or taboo in some years past. These diseases have been given some baseline evidence these days due to research findings on the use of chemicals in agriculture, manufacturing, transportation etc., (Appiah-Num, 2004; Asamuah Frimpong, 2013; Obiri-Danso *et al*., 2008).

A study by Oppong-Anane and Apori, (2007), indicated that, meat and hides from cattle, goat and sheep were widely consumed by Ghanaians, for providing proteins that build up and repair worn out tissues in the body.

Obiri-Danso *et al*. (2008), however reported that the various ways of rearing, slaughtering or killing, processing and preservation of the carcasses or hides for consumption, as well as handling the meat product itself tend to contaminate the meat if done in an unprotected or unhygienic environment. Unfortunately what these unsuspecting consumers probably receive is a cocktail of toxins and contaminants, which have made their way into their food and meat sources. These toxins may biologically accumulate in the tissues of their consumers and pose as carcinogen to human health risk, such as cancer and or death (Helferich and Winter, 2001). That is, if people are exposed to toxic pollutants at sufficient concentrations and for enough duration, they may increase their chances of getting cancer or experience other serious health effects.

1.6: Objective

The main objective of this study was to assess the heavy metals and microbial quality of cattle hide (welle) and beef respectively in Tarkwa Municipality.; Western Region.

1.7: Specific Objectives

The specific objectives were to: ○ determine the level of heavy metals (Pb, Cu, Fe and Zn) on singed and unsinged cattle hide in available at selected retail markets in Tarkwa Municipality.

- determine the microbial quality of raw beef available in some retail markets in Tarkwa Municipality.
- determine the microbial quality of meat cutting equipments and its environment in selected retail outlets in Tarkwa Municipality.
- identify microbial pathogens on meat sold in retail outlets in Tarkwa Municipality.
- examine the microbial changes due to environmental temperatures and pH by post-slaughter timings.

1.8: Significance of the Study ○ The study will highlight on the various measures of processing and preservation, for consumption which will serve as the basis for implementing managerial practices so as to prevent health risk effects such as diseases.

- Having knowledge about the extent of microbial contamination in certain meat will contribute greatly in formulating policies on meat products to farmers, butchers as well as consumers. ○ The study will also assist in monitoring as well as assessing the effects of potential impact of heavy metals accumulation in hide and its associated health risk impacts to consumers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat

Lawrie and Ledward (2006), defined meat as animal flesh (skeletal muscle plus any attached connective muscle tissue or fat), that is eaten as food. Humans are omnivores and have hunted and killed animals for meat since prehistoric times. However, domestication of animals such as chicken, sheep, pigs, goats and cattle, and eventually their use in meat production on an industrial scale has been as the advent of civilization (Dhanda, 2001). Meat is mostly the muscle tissue of an animal which is the main edible part of domestic mammals.

Gracey *et al.* (1999), also defined meat as the animal tissue used as food, mostly skeletal muscles and its associated fats but it may also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood. Raw meat generally refers to any type of uncooked muscle tissue of an animal used for food. In the meat production industry, the term 'meat' refers specifically to mammalian flesh, while the word 'poultry' and 'seafood' are used to differentiate between the tissue of birds and aquatic creatures respectively (Smil, 2002).

Scanga *et al.* (2000) also reported that, animal muscle is roughly 75% water, 20% protein and 5% fat, carbohydrates and assorted proteins.

2.2 Consumption of Raw Meat

In meat societies, argument and debate have arisen over the ethics of consuming animals as food. Two of the main ethical objectives have to do with (i) the unnecessary killing of sentience (i.e. feelings) living beings, and (ii) the inhumane agricultural practices underlying the production of meat. Among the reason for objecting to the breeding and killing of animals for human consumption are concerns about animal welfare, animal rights, environmental ethics and religious scruples.

Altekruse and Sean, (1999) reported that consumption of meat had increased significantly all through the late 1990's, with an estimate of the global average in 2000 to be 38 kg / capita. The studies therefore projected that; meat production would be doubled by 2050 due to population growth. While the global meat industry provides food and a livelihood for billions of people, it also has significant environmental and health consequences for the planet. Experts predicted that the worldwide consumption of pork, beef and poultry and other livestock products will double by 2020, although this prediction may be dampened by the recent economic downturn (FAO, 2001). These trends will have major consequences on the global environment. Consequences for the environment may result from changes in the amount of production to meet changes in demand for consumption. It has also been projected by FAO (2001), that global meat consumption may double from 2000 to 2050, mostly as a consequence of increasing world population, but also partly because of increased per capita meat consumption (with much of the per capita consumption increase occurring in the developing world. Global production and consumption of poultry meat had been growing at more than 5% annually (FAO, 2001). Trends vary among livestock sectors. For example, global per capita consumption of pork had increased (almost entirely due to changes in consumption within China), but global per capita consumption of ruminant meats had been declining according to FAO's (2001) report. The consumption of meat shall result in vast transfers of 'virtual' energy, water and nutrients that shall occur among nations, eventually having large impacts on local and distant environments. A full accounting of the trends and projections will give us the capacity to propose policies to mitigate or ameliorate the negative aspects of these developments and position in order to address the multiple consequences of industrialized animal production systems. The global meat industry would be able to provide food and a livelihood for billions of people, notwithstanding its significant environmental and health consequences for the planet. Ironically, experts' prediction on the doubled meat production by 2050, may be delayed by the recent economic downturn.

According to Lund and O'Brien (2011), the risk of disease from ingesting pathogens found in raw meat is significantly higher than cooked meat, although both can be contaminated. Meat to be consumed can be incorrectly or insufficiently cooked, allowing disease-carrying pathogens to be ingested. Also, meat can be contaminated during the production process at any time, from the slicing of prepared meat to cross-contamination of food in a refrigerator. All of these situations, according to Lund and O'Brien (2011), lead to a greater risk of disease.

2.3 Quality of Meat

The quality of raw meat can be defined as the suitability of meat for use in a specified product (Bastin, 2007; Hozza *et al.*, 2014). If the meat is well suited for the product it is intended for, then the 'consumer' meat quality is defined as good. However, if the meat is less suitable for the product, then the 'consumer' meat quality is defined as poor. However, price and quality are key factors for success in food markets. These are important for the competitiveness and economic efficiency of firms and the whole supply chain in meeting demands.

Generally, meat is referred to as red or dark and white or light meat. White meat consist of muscles with fibres that are referred to as fast fibres, since they are used for quick burst of activity such as running and fleeing from danger. White or light meat refers to the lightercoloured meat of poultry as compared with dark meat like beef and some types of game (United States Department of Agriculture, Retrieved 2014-09-14).

Dark meat, which avian mycologists refer to as ‘red muscle’ is used for sustained activity-chiefly for walking, in the case of chicken, cattle, sheep or goats. Dark meat is mainly made up of muscles with fibres that are called slow fibres since they are used for extended periods of activity that need consistent energy source. Othman (2006) reported in his studies that, this energy is derived from fat cells which required oxygen for constant activity. The dark colour comes from the protein myoglobin, which plays a key role in oxygen uptake within cells. White muscle, in contrast, is suitable only for short, ineffectual bursts of activity such as, for chickens, flying.
Thus the chicken’s leg and thigh meat are dark while its breast meat (which makes up the primary flight muscles) is white. Although ducks and geese are considered as poultry, they have red muscles (and therefore dark meat) throughout since their breast muscles are more suitable for sustained flight. Dark meat contains high BV-proteins (essential micronutrients) and essential omega-3 polyunsaturated fats that are needed for good health throughout life (Othman, 2006). Prescott *et al*. (2002) indicated that though the nutritional composition of red meats may vary somewhat according to breed, feed regime, season and meat cut, in general lean red meat has a relatively low fat content, moderate in cholesterol and rich in protein and many essential vitamins and minerals.

The eating quality of lamb includes traits such as meat and fat colour, pH, tenderness and factors affecting the eating experience such as taste, juiciness and smell (Hozza *et al.,* 2014). Meat as being tender, appealing to the eye, wholesome and safe for consumption, nutritious and affordable are the qualities and factors, consumers look for in their choice (Brewer, 2011).

Meat quality is normally defined by the compositional quality (lean to fat ratio) and the palatability factors such as visual appearance, smell, firmness, juiciness, tenderness and flavour.
Therefore, it can be concluded that, the nutritional quality of meat is objective yet "eating" quality, as perceived by the consumer, is highly subjective (Brewer, 2011). These factors include carcass maturity, the amount and distribution of marbling within the lean. Beef carcass quality grading is based on degree of marbling and degree of maturity (USDA, 2012. *Accessed 2014/09/14*).

According to Adzitey, *et. al*. (2011) live weights before slaughter influence the quality of meat. Adegoke and Falade (2005) reported that, nutritive quality attributes of meat, include the nutrient content, nutrient availability and caloric value. The quality and quantity of protein in meat is reported to be high. However, there are considerable differences between the preferences of individuals including preference for different cuts of meat, lean or fatty, muscle or organ meats, methods of cooking, etc.

2.4 Nutritional Quality of Meat

The nutritional quality of meat is important to consumers with vitamin and essential mineral content (such as iron) and types of fatty acids (such as omega -3) playing key roles.

Meat products contain traces of several different vitamins. These include vitamin E, and vitamins B1, B2, B3 and B6. Vitamin E serves is an antioxidant that helps to stop free radicals from damaging cells. All B vitamins work together to convert food into energy. Vitamin B1 helps promote healthy muscles, nerves and a healthy heart. The human body uses vitamin B2 to manufacture red blood cells. Vitamin B3 helps promote good digestion. Also the body use vitamin B6 to manufacture protein.

Meat products contain several minerals including magnesium, iron and zinc. The human body needs magnesium to build and maintain strong bones. Transportation of oxygen through the blood is by the aid of iron. Zinc is important for maintaining a healthy immune system. Other nutrients that can be found in red meat include vitamin D and K, chromium, copper, folic acid magnesium, potassium, and selenium and n-3 fatty acids. The nine amino acids that the body cannot make are found in meat, thus making it a complete protein (Bastin, 2007 and Hozza *et al.*, 2014).

Meat is composed of water, protein and amino acids, minerals, fats and fatty acids, vitamins and other bioactive components, and small quantities of carbohydrate (USDA 2012; *Retrieved 14/09/2014*). Although lean meat has high water content, about 75% it is good source of protein 30 – 20% on a wet-weight basis compared with 8-12% in cereals (FAO, 1985).

According to Schmid and Walther (2013), the highest values of vitamin D are found in fish and especially in fish liver ranging from □ 2□*g*/*kg* to 477□*g*/*kg* and up to 1200□*g*/*kg*, depending on fish species and locations. They further concluded in their studies that offal provides considerable amounts of vitamin D up to140□*g*/*kg*, whereas the content in muscle meat is generally much lower (up to10□*g*/*kg*). The vitamin variations between meat cut and species could be observed by concluding that, the vitamin D concentration of egg yolk, up to57□*g*/*kg*, indicated values between the vitamin D contents of meat and offal. Processing does not influence the concentrations of vitamin D in meat and dairy products very much because of the fact that, vitamins are heat and oxygen tolerant (CFSAN, 2013; Retrieved 2014/10/08). However, exposure to light can significantly reduce vitamin D content.

Nutrition societies often advocate an intake of 3 portions of dairy and 1 part of meat, fish or eggs per day (CFSAN, 2013. Retrieved 2014/10/08). Schmid and Walther (2013) anticipated that, by complying with these recommendations, the maximal intake of vitamin D through animal food would be 3□*g* (diary plus meat), 7□*g* (diary plus eggs), and 49□*g* (dairy plus fish), per day. In addition to these essential vitamins, beef also offers vitamins E, and K. These vitamins are used in various important body functions. The other important minerals also found in beef, besides iron includes potassium, calcium, zinc and magnesium. These minerals are important for many essential body functions, including muscle, bone and cellular functions. Liver is by far the richest of animal tissues in all vitamins, and includes unchanged carotene as well as being the only tissue to contain more than a trace of vitamin D (CFSAN, 2013. Retrieved 2014/10/08). Reinmuth's (2010) report on red meat suggests that, red meat is a good source of both vitamin B12 and iron and eating moderate amounts of red meat eventually minimizes one's chances of becoming anaemic. Brewer (2011) also reported that 90% of consumers indicate taste as a major factor in quality meat selection, because several daily meat consumers perceived pork to have better taste, healthier and tender that do less frequent meat consumers. Therefore, the health benefits associated with eating low fat meat-products as well as the concepts of freshness and taste need to be incorporated into any new promotional campaign to meet the new trend in consumer preference as suggested by Wolfe (1998).

2.5 Indicators of Meat Quality

Meat is one of the most nutritious foods that humans can consume, particularly in terms of supplying high-quality protein (essential amino acids), minerals (iron) and essential vitamins. The longer a meat product is fried, the more its liquid content lost and tougher it becomes. The more tender the meat, the more rapidly juices are released by chewing and the less residue remains in the mouth after chewing.

The appearance (colour) of cooked or raw meat is important because consumers associate it with the product's freshness as they decide whether or not to buy the product based on their opinion of its attractiveness (Adegoke and Falade, 2005). The factors that lead to discolouration of meat includes the amount of haemoglobin and myoglobin that are present in the meat, the chemical state of the pigments, or the way in which light is reflected off the meat. Proper sanitation also helps maintain meat colour, which leads to more sales and reduced colour costs. The colour of freshly cut meat is purplish-red. After it is cut, meat "blooms" by absorbing oxygen from the air, turning bright red. Continued exposure to air causes the meat to turn brownish-red or greyish red.

The length of time between the initial 'blooming' and discolouration depends on several factors. Two of these factors, the presence of oxygen from the air and dehydration or loss of water from the surface, are controlled by proper packaging materials. Fresh meat packaging films, when properly used, permit oxygen to enter the package and act as a barrier to moisture loss. Two other factors, temperature and microbes, must be controlled by practices and operations in the retail market. According to Guerreso *et al.* (2013), there are several factors that affect the quality of meat. However, the main factors are nutrition, social environment, cooking, ageing time, post slaughter handling, slaughter procedure, pre slaughter handling, lairage time, lairage conditions, animal handling, disease, transport, slaughter weight, climate and genetics.

The energy required for muscle activity in the live animal is obtained from sugars (glycogen) in the muscle. In the healthy and well-rested animal, the glycogen content of the muscle is high. After the animal has been slaughtered, the glycogen in the muscle is converted into lactic acid and the muscle and carcasses become firm (rigor mortis). This lactic acid is necessary to produce meat, which is tasteful and tender, of good keeping quality and good colour. If the animal is stressed before and during slaughter, the glycogen is used up, and the lactic acid level that develops in the meat after slaughter is reduced. This will have serious adverse effects on meat quality.

Dark Film and Dry (DFD) meat can be found in carcasses of cattle or sheep and sometimes pigs and turkeys soon after slaughter. The carcass meat is darker and drier than normal and has a much firmer texture. The muscle glycogen has been used up during the period of handling, transport and pre-slaughter and as a result, after slaughter, there is little lactic acid production, which results in DFD meat. This meat is of inferior quality as the less pronounced taste and the dark colour is less acceptable to the consumer and has a shorter shelf life due to the abnormally low pH value of the meat (6.4 – 6.8). DFD meat means that the carcasses were formed from an animal that was stressed, injured or diseased before being slaughtered.

Pale Soft Exudative (PSE), meat in pigs is caused by severe, short-term stress just prior to slaughter. The stress could take place during off-loading, handling, holding in pens and stunning. Here the animals are subjected to severe anxiety and fright caused by man-handling, fighting in the pens and bad stunning techniques. All this may result in biochemical processes in the muscle, in particular the rapid breakdown of muscle glycogen. This can lead to the meat becoming very pale with pronounced acidity (pH value of 5.4-5.6 immediately after slaughter) and poor flavour. This type of meat is difficult to use or cannot be used at all by butchers or meat processors and is wasted in extreme cases. Allowing animals to rest for one hour prior to slaughter and quiet handling will considerably reduce the risk of PSE (Adzitey *et al.,* 2011).

2.6 Spoilage of Meat

Spoilage of meat occurs, if the meat is untreated, in a matter of hours or days and results in the meat becoming unappetizing, poisonous or infectious. Spoilage is caused by the practically avoidable as well as unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, by the people handling the meat and by their implements. Meat can be kept edible for a much longer time-though not indefinitely – if proper hygiene is observed during production and processing and if appropriate food safety, food preservation and food storage procedures are applied (CFSAN 2013; Retrieved 2014/10/08).

It is necessary for animals to be stress and injury free during operations prior to slaughter, so as not to unnecessarily deplete muscle glycogen reserves. It is also important for animals to be well rested during the 24-hours so that the glycogen levels in the muscles of the slaughtered carcass are as high as possible to develop the maximum level of lactic acid in the meat. Lactic acid in the muscle has the effect of retarding the growth of bacteria that have contaminated the carcass during slaughtering and dressing. These bacteria cause spoilage of the meat during storage, particularly in warmer environments, and the meat develops off-smells, colour changes, rancidity and slime. This is spoilage, and these processes decrease the shelf life of meat, thus causing wastage of valuable food. If the contaminating bacteria are those of food poisoning type, the consumers of the meat become sick, resulting in costly treatment and loss of man power hours to the national economies. Thus, meat from animals, which have suffered from stress or injuries during handling, transport and slaughter, is likely to have a shorter shelf life due to spoilage. This is perhaps the biggest cause for meat wastage during the production processes (FAO, 1985). The most important cause of discolouration is the action of microbes (bacteria, molds and yeasts) growing on the cut surface (Meat Inspectors Manual, 2007). Muscle, fat and bone in a living animal are particularly free of microbes. It is during the slaughtering, handling, cutting and packaging that the meat becomes contaminated. Meat spoilage by micro-organisms can manifest itself depending on oxygen availability (Table 2.1) Lawrie and Ledward (2006).

Table 2:1 Indicators of Aerobic And Anaerobic Spoilage of Meat.

Oxygen	**Microbial agent**	**Symptoms**
Present	Aerobic bacteria	Surface slime Discolouration Gas production Change in odour Fat decomposition

Present	Yeasts	Surface slime Discolouration Change in odour and taste Fat decomposition
Present	Molds	Sticky and "whiskey" surface Discolouration Change in order Fat decomposition
Absent	Anaerobic bacteria	Putrefaction and foul odours Gas production Souring

Source: Table adapted from Lawrie and Ledward, 2006, 166. *Retrieved on January 29, 2014.*

Extremely perishable meet provides favourable growth conditions for various microorganisms. Meat is also very much susceptible to spoilage due to chemical and enzymatic activities. The breakdown of fat, protein and carbohydrates in meat can result in the development of off-odours, off-flavour and slim formation which make the meat objectionable for human consumption. It is, therefore, necessary to control meat spoilage in order to increase its shelf life and maintain its nutritional value, texture and flavour. International management agencies, especially the World Health Organization (WHO), Food and Agriculture Organization (FAO) and International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures as HACCP and Good Manufacturing Practices (GMPs).

2.7: Microbial Food-borne Illness

Foodborne illness (also foodborne disease and foodborne poisoning) is any illness resulting from the consumption of contaminated food, pathogenic bacteria, viruses or parasites that contaminate food (US CDC: Food Poisoning Guide) as well as chemical or natural toxins such as poisonous mushrooms. WHO (2009) reports that, foodborne diseases encompass a wide spectrum of illnesses and are growing public health problem worldwide. They are the result of ingestion of foodstuffs contaminated with microorganisms or chemicals. The contamination of food may occur at any stage in the process from food production to consumption ('farm to fork') and can result from environmental contamination, including pollution of water, soil or air.

Symptoms vary depending on the causes, and type of microbiological organisms. A few broad generalizations can be made e.g. the incubation period ranges from hours to days, depending on the type of microorganism and on how much of contaminated food is consumed. Symptoms often include vomiting, fever and aches, and may include diarrhoea (CFSAN 2013; Retrieved 2014/10/08).

More than 40 different foodborne microbial pathogens are known to cause human illness, including bacteria, parasites, viruses, fungi and their toxins (US CDC, 2014: Food Poisoning Guide). The ecology of pathogens varies. Some pathogens such as *Listeria monocytogens*, are pervasive in the natural environment and may contaminate food during production or distribution. Other authors have found new ecological niches, such as *Salmonella serotype, Enteritidis* in eggs. The CDC has estimated that these elusive, unknown pathogens account for 81% of the foodborne illness in the United States (Mead *et al*., 1999). These unknown pathogens probably account to some extent for microbial epidemiologists' inability to identify the pathogens that caused over two-thirds of the 2,800 mass outbreaks of foodborne illness reported to the CDC during 1993-97. Since 2000, WHO and FAO have worked on a programme of activities with the objective of conducting risk assessments for the Codex Alimentarius

Commission and member countries (WHO, 2013). This programme is known as the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA). The key objective of JEMRA is to be in the forefront of the development of risk based approaches for the management of public health hazards in food, in order to mitigate the high levels of foodborne morbidity and mortality in the general population but especially for at-risk groups (infants, children, the elderly and the immune-compromised).

The organisms may infect the animal while still alive (endogenous disease) or may contaminate the meat after its slaughter (exogenous disease) (Lawrie and Ledward 2006). The study further reported that, there are numerous diseases that humans may contract from endogenously infected meat, such as anthrax, bovine tuberculosis, brucellosis, salmonellosis, listeriosis, trichinosis or taeniasis.

2.7.1 Foodborne Pathogens

Every year in the United States, 6.5 million to 33 million cases of illness are diagnosed due to microbial pathogens, with about 9000 deaths occurring annually as well (Ahn *et al*., 2000). According to a multi-state study published in the American Journal of Preventative Medicine, the annual cost of disease caused by foodborne pathogens is estimated to be anywhere from 9.3 to 12.9 million dollars in medical costs and productivity losses (Altekruse and Sean (1999). According to Duffy *et al*. (2001), the main source of disease caused by microbial pathogens is usually raw meat. The type of pathogen present varies depending on the type of meat eaten. For instance, the most common pathogen found in beef is *Escherichia coli* (Omorodion and Odu; (2014) while *Salmonella serovars* are more common in poultry (Duffy, 2001). Some diseasecarrying pathogens found in beef are *Salmonella*, *Escherichia coli*, S*higella*, *Staphylococcus aureus*, and *Listeris monocytogenes* (Duffy 2001; Myint *et al.*, 2006; Scanga *et al*., 2000). The symptoms of an *E. coli* infection include bloody diarrhoea, severe abdominal pain, and possible complications for the immuno-compromised, elderly or children. These complications can include Haemolytic Uraemic Syndrome (HUS) and neurological problems (Lund and O'Brien (2011). Altekruse and Sean, (1999) also reported that frequent fevers, bloody diarrhoea, long duration of illness and hospitalization are all symptoms of *Vibrio gastroenteritis*. While most of these diseases are still most commonly found in raw meat, the instances of detection in other media are. For instance, *Salmonella* is most commonly found in poultry, but has been recently identified in sources such as eggs, diary, meat, fresh vegetables and fruits (Duffy 2001).

According to a study published in the Journal of Food Safety in Consumer Attitudes and Awareness of Disease, most outbreaks of food borne illness result from contaminated raw foods, cross-contamination, insufficient cooking, inadequate cooling or a lapse of more than 12 hours between preparing and eating (Sammarco *et al*., 1999). The study focused on habits of consumers in the kitchen with preparing raw meat. According to the results, 14% did not wash utensils or dishes between using them for raw and cooked foods, and 75% were unaware of the risks of storing raw meat on upper levels of refrigerators and the risk of cross-contamination. The instances of Salmonellosis and Campylobacteriosis have increased in the past decade, according to the study, most frequently because of the improper handling of foods by consumers and food service workers. Data gathered in a multi-state survey in 1999 found that out of 19, 356 adults interviewed, 19% did not wash hands or cutting boards after handling raw meat, 20% ate pink hamburgers often, 50% ate under cooked eggs on a regular basis, 8% had raw oysters habitually, and 1% drank unpasteurized milk (Altekruse and Sean, 1999). They further concluded that, these behaviours increase the risk of acquiring *Salmonellas*, *Vibrio vulnificus*, *Vibrio gastroenteritis* and *Escherichia coli*.

Faecal matters has been a major source of contamination and could reach carcasses through direct deposition, as well as by indirect contact through contaminated and clean carcasses, equipment, workers, installations, and air (Gurmu and Gebretinasae, 2013). Abdalla *et al*. (2009), in their studies indicated that, there were significant increases in total bacterial counts at skinny points than that at washing operations; also, dirty workers hands, clothes and equipments of the slaughter house acted as intermediate source of contamination of meat.

2.8 Animal Hide

A hide is an animal skin treated for human use. Hides include leather from cattle and other livestock animals, alligator skins, snake skins for shoes and fashion accessories and furs from wild cats, mink and bears. A living animals uses hide for several functions including protecting the body from injuries, climatic, bad weather and environmental influences, and body temperature regulation (LANXESS, 2010). Raw hides or skins are mostly provided as byproduct of the meat from farm animals in Ghana. In some West African countries such as Ghana, hides/leather is produced on a domestic or small industrial scale, but most leather making is done on a large scale (Akwetey *et al*., 2013; Obeng *et al.*, 2013; Adzitey *et al.*, 2011; Teye and Salifu, 2006).

An animal's hide is a stretched, dried, tanned and flexible material. ICT (2010) indicated that, the tanning industry and the downstream industries such as footwear, furniture, automotive, clothing, leather goods and saddler are entirely dependent for their raw material on suppliers of cattle hides, sheep skin and small number of goat and other skins. Fur and hides find their main use today as clothing, particularly costs.

Following the international financial crises during much of 2000's, the global hides and skins market was deeply affected by the widespread economic downturn (Table 2.6) (FAO, 2001).

Events began when, towards the end of 2008, markets for finished products were hit hard by a reduction in orders of hide. The abrupt slowdown in global leather purchases and bleak prospects for demand was especially felt by important buyers of leather and related products, such as the shoes, the automobile and the furniture industries. As a result, raw material prices fell, prompting a decline in world hides production in 2008 by around 2%, with much of the contraction concentrated in developing countries. However, outlook for global production in 2009 has brightened slightly, with developing countries likely to lead a moderate recovery. Asian and Latin American countries have dominated the dynamic of production in recent years; while European output markets have shrunk considerably (Table 2.2).

Table 2.2 Production of Hides (1000 tonnes)

BOVINE	2002/04 average	2005	2006	2007	2008	2009
World total	6011.2	6099.3	6159.8	6321.6	6195.6	6279.5
China	756.6	862.4	862.4	908.4	754.6	882.1
USA	895.1	813.4	847.7	860.0	867.2	833.6
Brazil	710.0	730.0	730.0	752.0	753.0	767.6
India	412.0	417.9	417.9	431.7	459.1	441.2
EU (25)	686.0	695.5	677.0	697.9	695.4	682.1
Argentina	352.5	403.2	403.2	421.3	421.9	425.6
Russian Fed	225.4	202.8	202.8	197.7	191.4	193.7
Developing countries	3445.1	3681.9	3710.2	3822.4	3710.2	3837.5

Developed countries	2566.1	2417.4	2449.6	2499.2	2485.3	2442.0
SHEEP	**2002/04 average**	**2005**	**2006**	**2007**	**2008**	**2009**
World total	384.2	408.8	417.7	425.9	426.8	410.2
China	64.9	80.7	84.1	89.7	90.3	94.0
European (15)	62.6	64.8	65.0	69.0	68.8	66.7
Australia	35.7	37.4	39.6	36.4	34.9	32.0
New Zealand	40.0	39.9	41.1	41.9	41.3	29.1
Iran	15.1	15.1	15.1	15.2	15.2	13.7
Indis	12.1	12.0	11.9	11.9	12.5	12.0
Developing countries	199.5	223.6	229.4	229.4	232.0	232.6
Developed countries	184.6	194.1	196.5	196.5	194.8	177.6
GOATS	**2002/04 average**	**2005**	**2006**	**2007**	**2008**	**2009**
World total	236.0	252.4	257.4	265.2	270.3	271.2
China	77.4	90.3	93.8	100.1	100.7	103.6
India	47.0	47.2	47.5	47.7	50.0	48.6
Pakistan	14.2	15.3	15.3	15.6	15.6	15.9
Bangladesh	13.7	13.7	13.7	13.7	14.5	14.2
Sudan	8.0	9.6	10.6	10.6	10.5	10.9
Iran	6.0	6.0	6.0	6.0	6.0	5.9
Developing countries	222.1	238.5	244.0	251.1	256.2	257.1
Developed countries	13.9	13.9	13.5	14.1	14.1	14.1

Source: Statistics Research and Information Directorate (SRID) MoFA Accra.

According to ICT (2010), the volume of hides and skins exported globally fell by five percent, but prices fell more dramatically and the global earnings from the export of raw hides and skins fell by almost 30% from 2008 to 2009.

It further reported that, a recovery in prices from mid-2009 was initially strong and by March 2011 Chicago hide prices had risen above levels that had prevailed through most of 2004 to 2008. Apart from a down turn in prices in late 2011, prices of raw hides and skins remained firm in 2012, surpassing their levels prevailing prior to the crisis. Developing countries, where tanning capacity rose fastest, reduced their exports of raw skins by an average of 1.3% per annum (Table 2.3) FAO (2001).

Table 2.3 Raw hides and skins production by Animal type and Economic Status-2000.

Countries/Type	Bovine hides (Tonnes)	Goat skins (Tonnes)	Sheep skins (Tonnes)
DEVELOPING COUNTRIES	17, 023, 284	3116450	3341516
Latin America	6377499	88,543	32600
Africa	2339824	565298	529781
Near East	855960	314150	979773
Far East	7550001	2148459	1505962
DEVELOPED COUNTRIES	13901673	132707	3233066
North America	4730164	0	52332
Europe	5760212	95010	1508120
Area of former USSR	2298297	26729	713425
Oceania	1113000	10968	959189

Source: FAO statistics (2001)

In Africa, countries such as Nigeria, Sudan and Kenya are much noted for the exportation of hides. A study by Mwinyihiya (2008) reported that in Kenya, the hides, skins and leather industry contributed an estimated 4.5% to agricultural gross domestic product (GDP). The report further stated that in the recent past in Kenya an economic survey showed a 10.3% growth in the leather sector. In Northern Ghana, hides and skin play an important socio-cultural role among many tribes. The hide/skin/leather serves as the official seat of the chiefs which signifies the wealth, prestige, power and authority, value and customs as well as tribal identification of the chiefs. (Oppong-Anane and Apori, 2007).

2.9 Consumption of Singed Cowhide

Singed cowhide "welle" is a delicacy used in the preparation of many Ghanaian dishes, including stews and soups. However, 'welle' has been described by health experts as unhygienic for human consumption, due to how it is prepared (*The Ghanaian Chronicle, August 25, 2011*).

Animal Health and Veterinary Laboratories Agency (AHVLA) (2012) reported that, where hides and skins are to be used for the production of gelatin and/or collagen for human consumption their storage must comply with the requirements for fresh meat in the food hygiene legislation. That is, they must have come from animals that have passed ante and post mortem inspection. In Ghana hides are processed into 'welle', a Ghanaian delicacy consumed by all social classes (Oppong Anane and Apori, (2007); Obiri-Danso *et al*., (2008); Okiel *et al*., (2009).

On November 14, 2000, BBC News reported that, the leather industry in Nigeria was going through difficult times. The report attributed these to the fact that, skin of animals like cows, sheep and goats, which were needed for the production of goods like shoes, bags and belts, were being eaten at an alarming rate. In Ghana slaughtered livestock such as cattle, sheep, and goat are usually singed to get rid of the fur and this eventually maintains the hide of the carcasses (FAO, 1985).

2.10 Hide, Heavy Metal Consumption and Health Issues

Heavy metals refer to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), Cadmium (Cd), arsenic (As), chromium (Cr), thallium (Ti), and lead (Pb).

The use of scrap car tyres as fuel for singeing carcasses has been reported by several authors (USFA, 1999; Okiel *et al.*, 2009; Jayasekara *et al*., 1992; Leita *et al*., 1991) to impart some toxic heavy metals to the singed skin, which could diffuse into the meat, thus rendering them unsafe for consumption, as it poses several health threats to the consumer. Obiri-Danso *et al.* (2008) reported high levels of heavy metal residues in goat and cattle hides singed with scrap tyres which made them unsafe for consumption.

The Institute of Medicine (IOM) put acceptable upper intake levels of Fe as 40 mg/day for infants and children of age 13 years, 45 mg/day for adolescent ages (14-18 years) and adults of 19 years and above 55 mg/day in 2001. 15 mg was the suggested regular daily intake (RDI) for zinc whereas doses levels >25mg may cause anaemia and copper deficiency (LWTPH, 2009). WHO also established a potential tolerable weekly intake (PTWI) value for Pb in adults as 3.0 *mg* (430 μg/day) (Appiah-Num, 2004).

Chronic low-level intake of heavy metals have damaging effects on human beings and other animals, since there is no good mechanism for their elimination. Metals such as lead, mercury, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic (Ellen *et al*., 1990).

As reported by Akan *et al*. (2010) heavy metals may enter the human body through inhalation of dust, direct ingestion of soil, animal products and consumption of food plants grown in metalcontaminated soil. Potentially toxic metals are also present in commercially produced foodstuffs.

Predicting exposure to potentially toxic metals from consumption of food is more complicated because uptake of metals by plants depends on soil properties, animal and plant physiologic factors, coupled with environmental conditions for its processing.

The symptoms that an acute oral Zn dose may provoke include: tachycardia, vascular shock, dyspeptic nausea, vomiting, diarrhea, pancreatitis and damage of hepatic parenchyma (Salgueiro *et al*., 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

With a total land area of 978.26 sq. km Tarkwa-Nsuaem Municipality is one of the 22 administrative MMDA's in the Western Region of Ghana as shown in Figure 3.1. The sampling was done in Tarkwa, the municipal capital. The district is located between 4^o5^1 and longitude 5^o5^1 and shares boundaries with Prestea-Huni-valley district to the north, Nzema East Municipality to the west, Ahanta West District to the south and Mpohor Wassa East District to the East (Ghana Districts retrieved January 26, 2014).

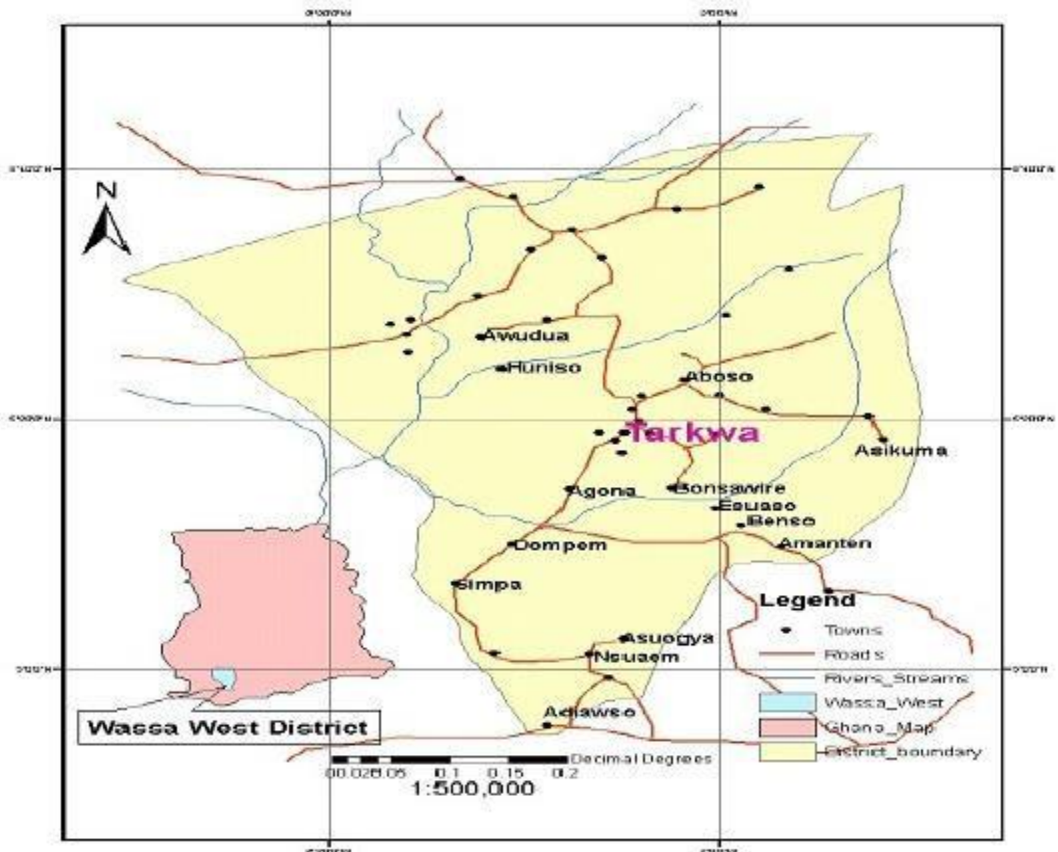

Figure 3.1: Map of Western Region showing Tarkwa Nsuaem Municipality.

About 66% of the entire active population is engaged in agricultural production (crop and livestock farmers) whilst the remaining 32% find themselves in the area of commerce, private informal sector and hospitality industries. Tarkwa is noted of mineral exploration and mining in Ghana. There were two main slaughter houses where animals are slaughtered for meat. Averagely, the combined-slaughter rate in Tarkwa is 1 or 2 for cattle and 2-8 for small ruminants (goat and sheep) per day.

3.3 Sample collection

Beef swab samples and cattle hides were obtained from four retail outlets in two well known and most popular meat and hide selling markets in Tarkwa namely: Central market (Layout) and Karikwanaano. A total of 192 replicated raw beef and its environmental swab samples (n= 384) and 24 unprocessed and processed cattle hide (firewood and scrap tyre singed) were randomly collected during the periods from December, 2013 to January, 2014 and from March to April 2014. These periods were chosen because of the large quantity of carcasses processed for sale during festive period such as Christmas, Easter as well as Eid-al-Fitr celebrations.

3.4 Measurement of Temperature and pH

Temperature and pH readings of raw beef were taken for times daily; early morning (6:00-8:00), afternoon (12:00-14:00) and late evening (16:00-18:00). Each period of reading was taken twice. Temperature and pH reading were taken from beef sold at central market and karikwanaano using compact digital thermometer and portable pH-meter for direct measurement in meat.

3.5 ASSESSMENT OF MICROBIAL QUALITY OF BEEF AND RETAIL OUTLET ENVIRONMENT.

3.5.1 Assessing Raw Beef

Samples were collected directly from the carcass within an hour post-slaughter and delayed market hours, in order to compare the microbial changes due to environmental temperatures, pH and post-slaughter timings. Averagely, fresh swab sampling and delayed swab sampling were done between 6am – 8am and 4pm – 6pm respectively. Two samples were collected from each sample type (surface swabs from beef, cutting equipment and surface swabs from environments) at each visit. Swab sample collections were replicated for 2 times each sample-type taken. Microbial collection were mainly done by surface swabs taken 15-20cm^2 of the surface of meatcutting equipment such as knives, wooden boards, weighing scales and carcasses meat. More so, surface swabs were taken from the surrounding environments such as the retail shops floors and workers hands. Collection was dependent on the size of the premises as well as on the cooperation of the shop owners.

Surface swab samples collection were taken aseptically using sterile swabs in 0.1% peptone water, neatly labelled (coded as Central Market fresh sample (CF), Central Market delayed sample (CD); and Karikwanaano fresh sample(KF), Karikwanaano delayed sample (KD), on glass tubes), were transported to Samartex Thaumatin microbiological laboratory within 2 hours in an insulated coldman boxes with ice packs for analysis. After collection, bacteriological analyses of the samples were performed to assess the selected microbial attributes such as total viable count (TVC), total coliform count (TCC) and total staphylococcal count (TSC) in cattle meat by using Plate Count (PC) agar, MacConkey (MC) agar and Staphylococcal Media (SM-
110) media to find out the microbial quality of beef in these retail outlets in the Municipality.

3.5.2 Enumeration of TVC, TCC and TSC

Swabs (pooled swabs) samples were added to 10ml of 0.1% peptone water and homogenized for 2 minutes. For the TVC and TCC determination, 0.1ml of each ten-fold dilution was transferred and spread on triplicate NA agar using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile wire loop spreader. One sterile spreader was used for each plate to prevent contaminations. The plates were then incubated aerobically at 37ºC for 24 hours before colonies were enumerated and reported as colony forming unit per cm^2 (cfu/cm^2).

The formula:

$$N = \frac{\sum c}{d\left(1 \times n_1 + 0.1 \times n_2\right)}$$

was used to calculate the number of colony forming unit per cm^2 where;

N = Number of colonies per cm^2

$\sum c$ = Sum of all colonies on all plates counted, n_1 = Number of plates in first dilution counted n_2 = Number of plates in second dilution counted d = dilution from which the first counts were obtained (dilution factor)

3.5.3 Microbial isolation and identification

Staphylococcus spp. were isolated and identified by first sub-culturing colonies on nutrients agar to obtain pure cultures. Morphological characteristics of the pure cultures, gram staining as well as other biochemical tests were then used to confirm the species.

Salmonella; the samples were pre-enriched in buffered peptone water (incubated aerobically at

37^{o}C for 24 hours), followed by enrichment in rappaport and vassiliadis broth (incubated aerobically at 42^{o}C for 24 hours) and plating on oxylose lysine deoxycholate agar (incubated aerobically at 37^{o}C for 24 hours).

E. coli identification, 1ml of the dilutions were inoculated on McConkay agar (Oxoid Ltd. Basingstoke, Hampshire, England) and incubated aerobically at 37^{o}C for 24 hours. Colonies that were suspected to be *E. coli* were isolated and confirmed using gram staining and other biochemical tests such as indole test, catalase test, methyl red, urease activity and sugar fermentation tests.

Streptococcus spp were identified by inoculating 1 ml of the dilutions on blood agar incubated at 37^{o}C for 24 hours. Colonies were then further identified using their morphological characteristics, gram staining and other biochemical test.

3.5.4 Confirmation of microbial isolates

Gram staining and biochemical tests such as catalase, oxidative-fermentative, mortality, methyl red, urease activity, coagulases, indole test, and gelatin hydrolysis were carried out to confirm the bacterial species. The manufacturer's instructions were used to prepare the various media.

3.5.4.1 Coagulase Test

Coagulase is an enzyme that clots blood plasma. This test is performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus.* Coagulase is a virulence factor of *S. aureus.* The formation of clot around an infection caused by these bacteria likely protects it from phagocytosis. This test differentiates *Staphylococcus aureus* from other coagulase negative *Staphylococcus* species.

Method: Two drops of fresh plasma were placed on two clean grease-free slides. The test organism was picked using a sterile wire loop and mixed homogenously with plasma and stirred for 5 seconds.

Reaction observed: A coagulase positive results was indicated by clumping that was not reemulsified.

3.5.4.2 Catalase Test

This test is used to identify organisms that produce enzyme, catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Method: A small colony of the culture was picked using a small wire loop and put into a test tube containing 2ml of hydrogen peroxide solution.

Results observed: Production of gas bubbles in the clean test tube indicated positive reaction.

3.5.4.3: Oxidative Test

This test is used to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative *Enterobacteriaceae* and oxidase positive *Pseudomadaceae*.

Cytochrome oxidase transfers electrons from the electron transport chain to oxygen (the final electron acceptor) and reduces it to water. In the oxidase test, artificial electron donors and acceptors are provided. When the electron donor is oxidized by cytochrome oxidase it turns dark purple. This is considered a positive result.

Method: Oxidase reagent (Tetramethyl P-phenylene-diamine) was placed on a clean filter paper. Using a sterile glass rod, the test organism was picked and homogenized on the filter paper containing the oxidase reagent.

Results Observed: Purple colouration produced within 5-10 minutes indicated positive results.

3.5.4.4: Sugar- Hydrolysis

Five grammes of glucose was weighed and dissolved in 250ml of distilled water. The mixture was boiled for 30 minutes and allowed to cool. Two drops of methyl red was added to the mixture and test organism inoculated. This was incubated for 48 hours and examined.

Results Observed: A deep red colouration indicated positive results.

3.5.4.5: Indole Test

Five grammes of agar was weighed into 2.5g of sodium hydroxide dissolved in 250ml of distilled water and autoclaved at 121ºC for 15 minutes. 15ml of the broth was then poured into a test tube and the test organism inoculated into the mixture. This was incubated at 37ºC for 2 days. A drop of Kovac's indole reagent was added and the tube gently shaken and allowed to stand. The set up was examined and a deep red-pinkish colour indicated a positive results.

3.6 ASSESSMENT OF HEAVY METALS IN HIDE

3.6.1 Processed hide collection

A total of twenty four (24) freshly singed cattle hides were obtained from four retail outlets on two retail markets; Central market (Layout) and Karikwanaano, in Tarkwa Municipal for the study. Out of this number, 12 cattle hides were singed-treated with scrap tyres (T) while the remaining 12 were singed-treated using firewood (F). The control for the study was taken from the un-singed carcasses before the singing took place. Approximately 300g of each hide singedtreated and unsinged were collected. The samples were labelled, packed in the ice chest containing ice packs and then transported to the Ghana Atomic Energy Commission (GAEC) Chemistry laboratory for chemical analyses. The samples were washed with distilled water and were oven dried to a constant weight at 100^{o}C for 24 hours. A meat grinder was used to milling the dried samples and digested for analysis. Both the singed-treated and un-singed cattle hides' samples were stored in a freezer at -20^{o}C before sample analysis.

3.6.2 Laboratory analysis of heavy metals.

The total lead (Pb), Copper (Cu), Iron (Fe) and Zinc (Zn) residues in the samples were determined using the procedures of the Association of Official Analytical Chemists (AOAC, 1990).

3.6.3 Statistical Analysis

SPSS Version 20 and GraphPad Prism 6 for the Graph analysis were used for the statistical analysis. The probability level of significant differences (*p- value*) between sample means was set at $p < 0.05$.

Means of microbial contents were compared with the US Centers of Disease Control & Prevention (CDC) Standards for Maximum Microbial Permissible Levels (MPLs) (CDC, 2014).

CHAPTER FOUR

RESULTS

4.1: Microbiological Load on Beef and Retail Environments

On the average 2.55±0.27 log_{10}cfu/cm², 2.06±0.22 log_{10}cfu/cm² and 1.57±0.17 log_{10}cfu/cm² of total viable count (TVC), total coliform count (TCC) and total staphylococcal count (TSC) were recorded respectively for all swab samples from retail outlets in Tarkwa. Microbial growth indicative by TVC, TCC and TSC were all significantly higher in the delayed swab samples compared to the fresh swab samples. Notwithstanding, the time of sampling had a strong effect on all microbial growth indicators or parameters analysed in this study. In general though statistically not significant, beef sampled at the Karikwanaano recorded higher microbial growth than those from the Central market. The effect of microbial growth by location of sampling was weak (Table 4.1).

Table 4.1: Microbial contamination characteristics of meat stratified by type of sample and retailed markets (log_{10} of cfu/cm^2)

Parameter	TVC	TCC	TSC
Mean	2.55±0.27	2.06±0.22	1.57±0.17
Type of Sample			
Fresh Swab Sample	1.36±0.21	1.10±0.16	0.87±0.13
Delayed Swab Sample	3.74±0.37	3.02±0.30	2.28±0.24
P-value	<0.0001	<0.0001	<0.0001
Effect Size (η^2)	0.4096	0.4020	0.3741
Retailed market			
Central Market	2.30±0.37	1.79±0.29	1.36±0.22
Karikwanaano	2.80±0.40	2.34±0.33	1.79±0.25
P-value	0.3687	0.2194	0.2008
Effect Size (η^2)	0.0176	0.0326	0.0353

Data is presented as means± standard deviation. p is significant at < 0.05. (η^2)-Eta square for effect size: $\eta^2 \leq 0.04$ – Weak effect, $0.04 < \eta^2 \leq 0.36$ moderate effect, $\eta^2 > 0.36$ strong effect. TVC- Total Viable Count, TCC- Total Coliform Count, TSC- Total Staphylococcal Count

The study recorded significant microbial growth differences across the various retail sale environments. Comparatively, the highest microbial growth was observed on the beef. The butchers' knives recorded the seconded highest TVC followed by the wooden boards and shop floor. The butchers' wooden boards, shop floor and hands were the second, third and fourth most significant area where TCC and TSC were recorded. The weighing scales recorded the least microbial contaminants (Table 4.2).

Table 4.2: Microbial contamination characteristics of various meat sale environments ($log_{10}$of cfu/cm^2)

Parameter	TVC	TCC	TSC
Beef	5.32±0.71	4.21±0.62	3.02±0.46
Knives	2.46±0.55	1.51±0.26	0.91±0.16
Wooden Boards	2.38±0.61	2.13±0.56	1.67±0.47
Weighing Scales	1.37±0.32	1.18±0.31	0.86±0.23
Shop Floor	2.00±0.25	1.84±0.27	1.65±0.23

Workers Hands	1.78±0.25	1.51±0.17	1.34±0.37
P-value	<0.0001	0.0001	0.0006
Effect Size (η^2)	0.4787	0.4408	0.3911

Data is presented as means± standard deviation. p is significant at < 0.05. (η^2)-Eta square for effect size: $\eta^2 \leq 0.04$ – Weak effect, $0.04 < \eta^2 \leq 0.36$ moderate effect, $\eta^2 > 0.36$ strong effect. TVC- Total Viable Count, TCC- Total Coliform Count, TSC- Total Staphylococcal Count

In general, the microbial contamination characteristics observed though not statistically significant were higher on all items surveyed from the Karikwanaano than that of Central market. The exception however was observed on the hands of the butchers, where higher microbial contaminants were observed among butchers from the Central market than that of Karikwanaano market (Appendix 4).

4.2: pH and Temperature Readings on Beef

The average pH reading of beef in this study was slightly acidic (6.88±0.78 cfu/cm²). The study observed significant differences in the pH readings of beef depending on the time of day measured. The approximately neutral pH of beef in the morning (6.89±0.03 cfu/cm²) increases in acidity during the day peaking in the afternoon (5.04±0.02 cfu/cm²) before dropping during the late hours (5.71±0.14 cfu/cm²) (Figure 4.1A). No significant difference ($p \geq 0.05$) in pH reading was recorded among the two different outlets of sales as indicated in figure 4.1C.

As seen in figure 4.1B, the temperature readings of beef were highest in the afternoon, followed by readings recorded in the late hours and lowest in the early morning. No significant temperature difference was recorded among the two different outlets of sales (Figure 4.1D).

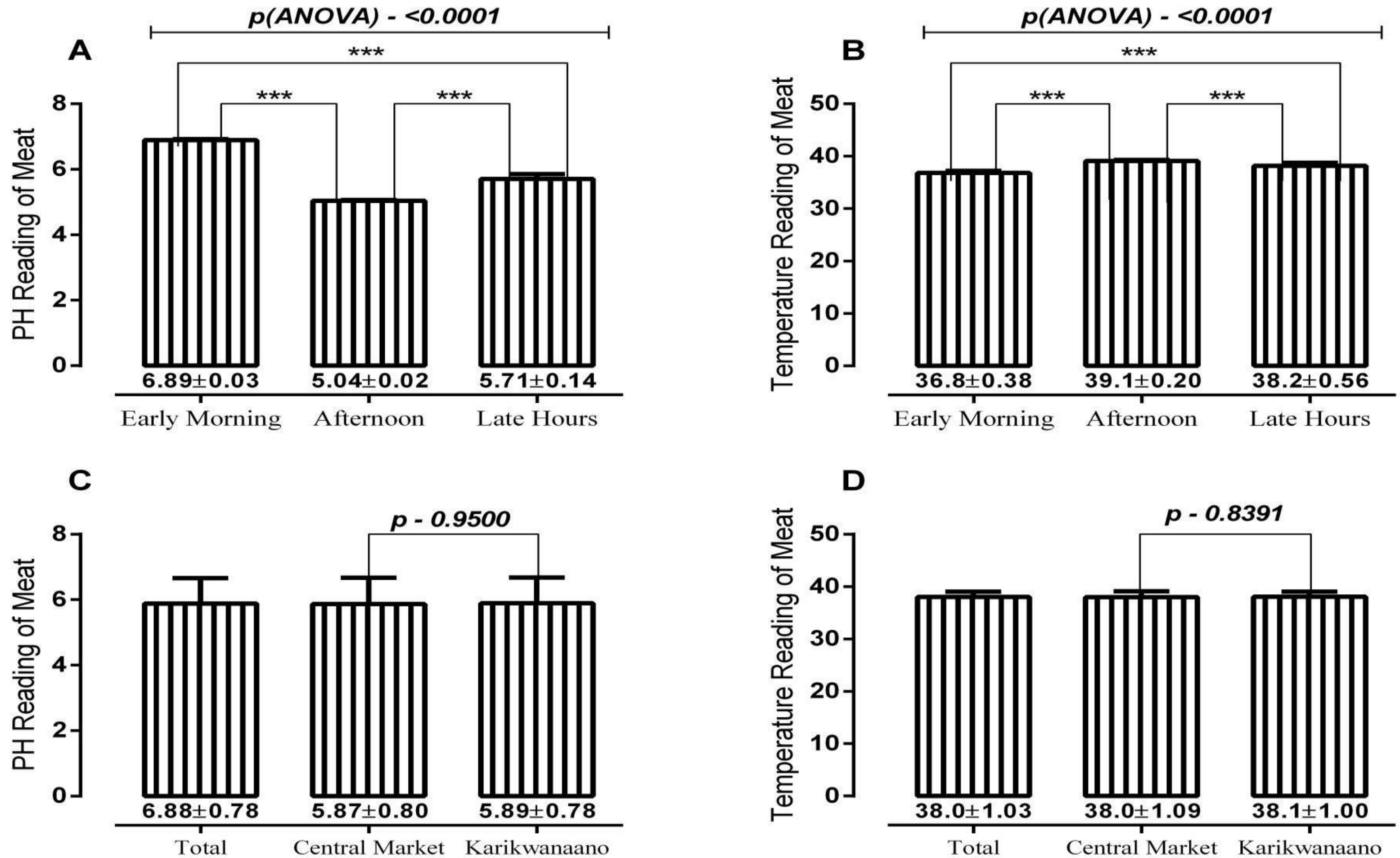

Figure 4.1: pH and Temperature recordings of meat stratified by time of day and retailed market

4.3: Heavy Metals Concentration in Hide

As shown in table 4.3, the average concentration of all metal contents in hide was lower than the permissible limit except for Lead (Pb). However statistical significant difference between permissible limit (50 mg/kg) and observed (23.44±5.70 mg/kg) was recorded only for Iron (Fe). The type of material used for hide processing had significant effect on the levels of metal content recorded in hide. Hide processed with scrap tyre recorded the highest level of Zn, Fe, Pb and Cu content compared with those processed with firewood. The unsinged hide had the least levels of metal content. In the case of Zinc (Zn), both the unsinged (15.79±1.27 mg/kg) and hide singed with firewood (26.94±2.45 mg/kg) recorded significantly lower concentrations than the permissible limit (50 mg/kg), whiles hide singed with scrap tyre (67.57±0.96 mg/kg), recorded concentrations significantly higher than the permissible limit (50 mg/kg). There was no significant difference on the level of Zn, Fe, Pb and Cu content of hide among the two retail markets.

Table 4.3: Metal content (mg/kg) in cattle hides stratified different processing methods and retail outlet (mg/kg)

Parameter	Fe (Limit -50)	Pb (Limit - 0.1)	Cu (Limit – 20)	Zn (Limit – 50)
Mean	23.44±5.70**	0.19±0.06	19.24±8.01	36.76±4.40
Processing Method				
Un-singed hide	9.11±4.70	0.02±0.01	10.47±2.23	15.79±1.27*
Firewood Singed Hide	22.86±5.40	0.23±0.10	20.15±4.38	26.94±2.45*
Scrap Tyre Singed Hide	38.37±5.39	0.31±0.13	27.10±4.13	67.57±0.96*
p-value	0.0250	0.6312	0.0461	0.0002
Effect Size (η^2)	0.9144	0.2642	0.8715	0.9971
Retailed Outlet				
Central Market	27.09±8.59	0.36±0.17	16.71±7.65	36.71±15.95
Karikwanaano	19.79±8.32	0.04±0.00	21.77±9.08	36.82±15.55
p-value	0.5745	0.1318	0.5014	0.9964

Data is presented as means ± standard deviation is significant at < 0.05. (η^2)-Eta square for effect size: $\eta^2 \leq 0.04$ – Weak effect, $0.04 < \eta^2 \leq 0.36$ moderate effect, $\eta^2 > 0.36$ strong effect. Fe- Iron, Pb-Lead, Cu -Copper, and ZnZinc.* significant different compared to permissible limit.

Different types of bacteria were isolated from all the swab samples from different retail outlets in Tarkwa Municipality. These bacteria identified included *Staphylococcus spp*, *Salmonella, Streptococcus spp., Klebsiella spp, Enterobacter spp and Escherichia coli.* *Staphylococcus spp., Salmonella* and, *Enterobacter spp* were isolated from the fresh swab samples in all markets except *Streptococcus spp* that was only isolated from Layout fresh swab samples.

Generally, bacteria that were isolated in the fresh swab samples were as well found in the delayed swab samples at the various retail outlets. *Escherichia coli* and *Klebsiella spp* were only present in delayed swab samples whiles *Klebsiella spp was* absent from Central market samples (Table 4.4).

The confirmatory tests using gram staining, sugar fermentation tests as well as other biochemical tests yielded the confirmation of gram negative bacteria *(Escherichia coli, Enterobacter spp, Klebsiella spp and Salmonella)* that are primarily potential pathogens (Appendix 2).

Table 4.4: The genera of bacteria isolated from swab samples from retail outlets in Tarkwa Municipality Meat Retail Outlet Bacteria Identified Fresh Swab Sample Delayed Swab Sample

Central Market	Staphylococcus spp.	Staphylococcus spp.
	Salmonella	*Salmonella*
	Enterobacter spp.	*Escherichia coli.*
		Enterobacter spp.
Karikwanaano	Staphylococcus spp.	Staphylococcus spp.
	Salmonella	*Salmonella*
	Streptococcus spp.	*Streptococcus spp.*
	Enterobacter	*Escherichia coli.*
		Enterobacter spp.

Klebsiella spp **CHAPTER FIVE**

DISCUSSION

The assessment of microbiological quality of beef and retail equipments; and heavy metals concentration in hides, were conducted mainly to determine the microbial loads on raw beef and its retail equipment, the level of heavy metals (Pb, Cu, Fe and Zn) on cattle hides singed with tyres and firewood, and to observe the sanitation and handling practices for fresh meat processing and retailing in some retail outlets at Tarkwa municipality.

5.1: Microbiological Quality

According to Jay *et al*. (2005), beef and its retail equipments offer an excellent growth media for a variety of microflora (yeast, bacteria and molds) as well as pathogenic organisms. This could be as a result of adequate moisture, pH, oxygen, temperature and large surface area of beef compared to other retail surfaces sampled and analyzed. The results of this study contradicts a study by Iroha *et al.* (2011) that stated that, traditional method of butchering using knives and cutting lines appeared more capable of minimizing faecal contamination than modern mechanized systems which are manned by a team of operators.

The presence of bacteria in beef and its retail equipment has been widely reported from different parts of the world (Jeffery *et al.*, 2003; Hassan Ali *et al.*, 2010; Gurmu and Gebretinsae, 2013; Kinsella *et al.*, 2008; Holds *et al.*, 2007). Gram negative bacteria has been isolated and implicated by researchers as an indicator of open air meat spoilage.

Though there are regulatory agencies and departments (Veterinary Service Directorate and Environmental Health Department) mandated to supervise and monitor the practice of good sanitary or hygiene in food handling, in order to ensure its wholesomeness, it was generally observed that majority of the butchers did not strictly adhered to Good Hygienic Practice (GHP) and Hazard Analyses and Critical Control Point (HACCP) Scheme. By virtue of inappropriate preventive measures observed at both slaughtering points and retail outlets of the study area, cross contamination was inevitable.

Though there were enumeration of microbial loads on all swab samples analyzed, delayed swab samples had significant difference in microbial loads and diversity than that of fresh swab samples. This large bacterial counts in delayed samples could be attributed to excessive handling of the meat and equipments, cross contaminations, duration of exposure as well as provision of favourable environmental conditions (optimum temperature, oxygen, moisture, nutrients) for microbial growth. This result of the study agrees with that of Siddiqui *et al.* (2008), who stipulated a swift increase of microbiological load in slaughter yards and meat stalls. Also the study results confirms that of Koffi-Nevry *et al.* (2011) who obtained higher microbial counts in late sales compared to early sales of beef offered for retail. Hassall (1995) also studied the microbial content of fresh meat from the time of slaughter to the retail outlet and concluded that bacterial count increased tremendously by the time the carcasses were received by the store. Generally, sampling time in this study had a stronger effect ($\eta^2 > 0.36$) on all microbial parameters measured (TVC, TCC and TSC). The prolong time the carcasses were openly displayed at the retail markets amidst high average ambient temperature (38.0±1.03°C) without any *in-situ* preservation could be the results of the high microbial loads on the samples. The low counts of microorganisms in fresh samples in this study is in agreement to the study by Adeyemo (2002) who reported that, animal products may be microbiologically contaminated by organisms living in them naturally or organisms entering from the surrounding during the carcasses processing operations. According to Adetunde *et al.* (2011) and Soyiri, (2008) more time raw meat stayed on stall shelf (displayed) shall lead to bacterial load builds up, hence deterioration rendering it unsafe for consumption.

Though not significantly different ($p > 0.05$) microbial loads (TVC, TCC and TSC) from Karikwanaano retail outlets were generally higher (2.80±0.40 *cfu/cm²*, 2.34±0.33 *cfu/cm²*, 1.79±0.25 *cfu/cm²*) than that of Central Market (2.30±0.37 *cfu/cm²*, 1.79±0.29 *cfu/cm²*, 1.36±0.22 *cfu/cm²*) respectively (Table 4). These differences in microbial loads count indicates that, the type of market or outlets had little influence on the level of microbial loads on samples but probably due to the time of sampling having the greater effect. This observation is not in line with Antwi-Agyei and Maaleku, (2014) who reported that there was significant difference between meat and fish products by market interactions on bacteria count in their study.

According to Meat Inspector's Manual, (2007) the use of contaminated water, unsterilized equipments (knifes, wooden boards, weighing scales, rusted hooks) are potential sources of contamination to cause food borne illness. It is therefore necessary for butchers and customers of meat products to adhere to stringent but good manufacturing practices to ensure food safety for human consumption. Detection of pathogenic microorganisms from meat cutting surfaces (knives, wooden boards, weighing scales and meat mincers) and retail surface swabs, has been reported by several authors (Myint, 2004; Hassan Ali *et al*, 2010; Eze and Ivuoma, 2012; Adzitey *et al.*, 2011). In a similar study, Eisel *et al*. (1997) higher microbial counts from tables, knifes and workers hands may be attributed to insanitary practices performed in the slaughtering and retail outlets, employee's ignorance, by poor personal hygiene and contaminated floors. The present results are in accord with the study by Jeffery *et al.* (2003) who revealed that workers' hands and the equipment were major sources of meat contamination. Food can be infected with microorganisms as a result of 'coughing' and 'sneezing' from those who handle and process these foods (Okonko *et al.*, 2008).

By the interaction with the butchers in the study area, majority of them did not have adequate knowledge on Good Hygienic Practices (GHP) as well as HACCP standards. Usually, their mode of cleaning was washing with water and/or without detergents. It was noted that, Central market butchers and sellers primarily cleaned the meat contact surfaces/utensils twice a day. Whiles those of Karikwanaano cleaned theirs less than twice a day. This may account for the increased microbial load at Karikwanaano retail outlets. Meats were handled with bare hands without any protective equipment in all the outlets studied during the study. It is interesting to note that, workers hands had a higher microbial load than weighing scales mainly due to excessive touching and handling with bare hands. The presence of bacterial pathogens on meat-processing equipment and associated surfaces contribute to the contamination of meat. Gracey *et al.* (1999) as well reported that meat mostly touched by bare hands stood to be associated with remarkable changes of being inoculated with coagulase positive Staphylococci. The results from this study agreed with that of Soyiri (2008); Abdalla *et al.*, (2009); Obeng *et al.*, 2013; Koffi-Nevry, 2011) that unhygienic practices and poor handling of beef by butchers were major causes of contaminated beef.

Comparatively, beef had the highest microbial loads of TVC, TCC and TSC (5.32±0.71 *cfu/cm²*, 4.21±0.62 *cfu/cm²* and 3.02±0.46 *cfu/cm²* respectively) and this could be attributed to adequate growth media conditions for microbes such as, moisture, pH as well as favourable temperature. This confirms Rao *et al.* (2009) report that meats have a high water content corresponding to the water activity of approximately 0.99 which is suitable for microbial growth.

International Commission on Microbiological Specification (ICMSF, 1985) and Ghana Standard Board (GSB, 2013) put viable counts of fresh meat tissue at < log 6.00 cfu/g and < log 4.00 cfu/g. Averagely, the results obtained in this study were within ICMSF and GSB limits for fresh swab samples but above the set standard for delayed swab samples. The higher level of total coliform on beef in the delayed sample might be due to faecal contamination of dressed carcasses which may occur as a consequence of direct contact with faeces or the surfaces / floor that have been contaminated with faecal matter, unsterilized knife and improper handling of the meat.

Averagely, the TVC, TCC and TSC of all Samples analyzed were 2.55±0.27 (log_{10} cfu/cm²), 2.06±0.22 (log_{10} cfu/cm²) and 1.57±0.17 (log_{10} cfu/cm²) respectively. There were enumerations of significant differences ($p < 0.05$) between the various sample types (beef, knives, wooden boards, weighing scales, shop floor and worker hands). The observed contaminations by virtue of the high microbial loads on these sample types agreed with Bogere and Baluka (2014) who found significant *Salmonella* loads on meat, and its environmental equipments. Studies by Ahmad *et al.* (2013) and Haque *et al.* (2008) all had earlier reported an increase in microbial loads on goat meat obtained from slaughter yards and subsequently in meat stalls at late market hours which is in-line with this current study.

5.2: Microbiological Diversity

The microbial diversity (differences in form and species) in samples from retail outlets observed in this study was an indication of poor hygienic conditions associated in the chain of carcasses handling, processing and transportation for sale.

Kinsella *et al.* (2008), Omorodion and Odu, (2014) and Adzitey *et al.* (2014) all reported the presence of bacteria in meat similar to gram negative bacteria, identified in this study (*Escherichia coli*, *Salmonella*, *Enterobacter spp* and *Klebsiella spp*). *Staphylococcus spp* and *Streptococcus spp* were the other forms of bacteria that were isolated from the samples in this study. These organisms have been reported in various infectious disease outbreaks in Ghana (CIA: World Factbook, 2014). Staphylococcocal food-borne disease (SFD) is one of the most common food-borne diseases worldwide resulting from the contamination of food by preformed *S. aureus* enterotoxins (Kadariya *et al*., 2014). Symptoms of SFD include nausea, vomiting, and abdominal cramps with or without diarrhoea.

Abudey *et al.* (2014) reported that, the presence of these organisms on beef and retail equipments could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity.

S. aureaus, *E.coli*, *Bacillus spp*, *Enterobacter spp*, and *Klebsiella spp* in meat pie and yoghurts were as well reported by Clarence *et al.* (2009).

The isolation of *Enterobacter spp* and *Salmonella* was an indication of poor hygienic conditions and contaminated water used during slaughtering and processing as reported by Okonko *et al.*

(2008). Thus improper handling and improper hygiene might lead to the contamination of ready-to-eat foods and this might eventually affect the health of the consumers. Hence it raises a lot of public health concerns to consumers.

Salmonella species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute life threatening febrile illness (CDC, Food Poisoning Guide). This disease is a cause for concern and a major public health problem in developing countries that is mainly transmitted through food or drink or water, contaminated with urine or faeces of infected people or chronic carriers.

Although *S. aureus* is an important pathogen on animals, one of the main sources of *S. aureas* infection in humans comes from humans themselves who can contaminate meat or even all meals during preparation (Goja *et al*., 2013)

Chaubey *et al.* (2004), enumerated coliform bacteria in majority of their meat samples and suggested that raw meat and meat produce should be handled under strict hygienic conditions and stored in cool places to avoid contamination and safe guard the health of consumers.

The fresh beef sold to the public in open markets was grossly contaminated with coliform bacteria as well as other bacteria. This work has revealed that fresh meat sold in Tarkwa Municipality is contaminated by both gram positive and gram negative bacteria.

This results of study present similar findings to Adzitey *et al.* (2011) and Singh *et al.* (2014) that found various levels and numbers of total bacteria count, *Streptococcus spp.*, *Salmonella spp.*, *Escherichia coli* and *Staphylococcus spp*., on beef sold at selected markets in Tamale Metropolis in the Northern Region of Ghana.

The organisms isolated confirmed Clarence *et al*. (2009) who reported that, gram negative bacteria accounts for approximately 69% of the cases of bacterial food-borne disease. The presence of these organisms in the beef is indicative of public health hazard and gives a signal of the possible occurrence of food borne intoxification and infections. This also implies meat is viable sources of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards.

Staphylococcus aureus, which is a normal flora of the body, indicates contamination from handlers. The organism can pass onto food during harvesting, processing or even storage (Eze and Ivuoma, 2012). It is the major cause of food poisoning known as staphylococcal food poisoning. The poisoning is caused by the ingestion of an enterotoxin produced, which is characterized by diarrhoea and vomiting (Frazier and Westhoff, 2004).

E. coli is an enteric organism and its presence is an indication of faecal contamination of the samples. This may be attributed to improper sanitary conditions during processing of the meat from the water supply, unsterilized utensils and contamination by flies. It causes gastroenteritis in infants and young children (Brooks *et al*., 2004). Enabulele and Uriah (2009) also isolated E. coli from raw beef, sheep meat and goat meat and identified the existence of resistant isolates that pose threat to public health.

According to Kinsella *et al.* (2008) cooking processes and hygiene can greatly reduce the microbial load in the fresh meat to harmless level. Undercooked meat products have caused many food poisoning incidents associated with E. coli which is present in the faeces, intestine and hide of healthy cattle from where it can potentially contaminate meat during slaughtering process (Duffy *et al*., 2001).

5.3: Effect of Temperature and pH on Beef

Mackey and Roberts (1993) reported a range of 30°C and 39°C for the internal temperature of carcasses once the slaughtering was completed. The results of this study confirm this assertion though slightly above body temperature (37°C) on the average. This could be the results of the carcasses being exposed to ambient temperatures for a long duration of time. It must be noted that, an increasing temperature resulted in a decrease pH. This observation from the study was in line with Lawan *et al.* (2011) who reported that a rapid decrease of pH values at higher temperature may burst the lysosomal membrane in which some cathepsins could hydrolyze specific myofibrillar proteins. The mean pH found in this study was slightly acidic (pH< 7.0). The physical activity of the animals for hours before slaughter, reduces glycogen concentration and plasma glucose levels below critical values and eventually leading to increased pH above critical range of 5.5 to 6.0 (Ndou *et al.*, 2011).

5.4: Heavy metals in hides

Comparatively, the average concentrations of all heavy metal concentrations in hides recorded were lower than European Commission's Standards for Maximum Permissible Levels (ECR, 2006) except for Lead (Pb) (Table 6). ECR (2006) stipulates that the permissible limit of lead should not exceed 0.1mg/kg (ppm) in cattle hide or beef muscle for human consumption. Lead is frequently the cause of accidental poisoning in domestic animals, especially cattle (Khalafalla *et al*, 2011).

Generally, the results of heavy metal residue in cattle hide was an indication that, singeing (scrap tyre or firewood) of the carcasses increased the heavy metal contents, but those singed with firewood had lower concentrations of heavy metals than scrap tyre singed hide. However, there were no significant difference in concentration between the processing methods except for Zinc (Zn) ($p<0.05$).

Generally, there was a stronger effect ($\eta^2 > 0.36$) of type of processing method on the heavy metal concentration except Lead (Pb) which had a moderate effect ($0.04 < \eta^2 \leq 0.36$). This observation contradicts the findings of Eremong *et al.* (2011) and Okiel *et al.* (2009); who reported decreasing levels of heavy metal residues in singed cattle hides.

Obiri-Danso *et al.* (2008) however, reported increased levels of some heavy metals, when goats and cattles hides were singed with scrap tyres. The authors reported that the concentrations increased further when the singed carcasses were washed with water. These reports are indications that mode of processing and the source of water for washing singed carcasses have direct effects on the heavy metal concentrations of the carcasses. Concentrations of Zn, Pb and Cu in scrap tyre singed carcasses (67.57±0.96 *mg/kg*, 27.10±4.13 *mg/kg*, 0.31±0.13 *mg/kg* and 38.37±5.39 *mg/kg*) were significantly above the recommended Maximum Permissible Levels (MPLs) of European Commission Regulations (ECR, 2006).

From the results singeing was not the absolute source of heavy metals in hide, however, there were clear indications that singeing of the carcasses increased their heavy metal contents in hide. This study results disagree with that of Eremong *et al.* (2011) and Okiel *et al.*, (2009) who reported decreasing levels of heavy metal residues in singed cattle hides.

Results from the study confirms that of Obiri-Danso *et al.* (2008) who reported increased levels of some heavy metals in goat and cattle hides singed with scrap tyres. Other authors had also reported a higher concentration of heavy metals in hides than the recommended

Maximum Permissible Levels (MPLs) of the European Commission Regulations (ECR 2006).

Drinking water, soil and feed are potential sources from which these heavy metal residues contaminate meat and meat products. Other authors had also reported that singeing is not the only means by which animals accumulate heavy metal residues. Obiri-Danso *et al.* (2008) and Okiel *et al.* (2009) all reported that, environmental conditions partially account for levels of heavy metals in cattle hide rather than the processing method used alone. During evisceration and washing, contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses.

According to WHO (1996), excessive dose of Pb in the body can result in 'Lead poisoning'. Pb affects the haematologic, renal and neurologic systems and there is no evidence for a threshold below which Pb has no adverse effect especially in children health (Goyer, 1993).

Zinc concentration was found to be highest in the scrap tyre singed hide (67.57±0.96 *mg/kg*). Just a little or much can cause harmful effect to humans such as health Zinc Pox, nausea and vomiting (ATSDR, 1998). The recorded Zn level in the study contradicts that of Akan *et al.*, (2010) who reported a lower concentration of Zn in meat and animal products.

According to Gautam and Irfan, (2011) when cupper (Cu) accumulates in the liver and brain in higher level of concentration it can cause Wilson's disease.

Generally, the levels of heavy metals concentrations reported in all the processed hides were quite similar to some reported cases of heavy metal contaminations in meat and other animal products in Ghana (Obiri-Danso *et al.*, 2008, Essumang *et al.*, 2007, Adam *et al.*, 2013).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1.1: Microbiological Quality of Beef

The high microbial load on the beef and its environmental equipment surfaces in this study underscores poor level of personal hygiene and poor sanitation right from the slaughter house to the retail outlets in the Mnicipality. This study showed poor microbiological quality of retail beef offered for sale in Tarkwa Municipality.By virtue of the high enumeration of pathogenic microorganisms in the current study, there is no doubt that, hygienic protocols were not followed by both butchers and sales men. These eventually contaminated the meat, rendering some unwholesome for human consumption. The study showed that meat sold to the public was contaminated with pathogenic bacteria. The presence of *Staphylococcus spp*, *Salmonella* *spp*, *Streptococcus spp* and *E. coli* is worrying because of their ability to cause diseases or viable source of various diseases. It is therefore appropriate for consumers to buy meat from the retail outlets early in the morning with less microbial load rather than the evening with unacceptable microbial loads.

6.1.2: Heavy Metals in Cattle Hides

A generally comparative assessment of the treatment processes showed that singeing of cattle carcasses with firewood and scrap tyres, increases the heavy metal concentrations in hides above the Maximum Permissible Limits. It can be deduced that scrap tyre singed cattle hides contains adequate heavy metal concentration that renders it as potential health risk when consumed and accumulated for a period of time. Environmental Inspectorate Division, Veterinary Services Directorate and all stakeholders must monitor and supervise the activities of local butchers in Ghana. This would ensure Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) schemes are observed.

6.2: Recommendation

This study has revealed that beef and hides sold from retail outlets in Tarkwa Municipality were contaminated with pathogenic microbes and heavy metals respectively. It is therefore appropriate to make the following recommendations for action by all stakeholders.

Retail Health Inspectors and the Veterinary Services Directorate (VSD) must monitor and supervise the processing, transportation, sale of beef and hides to enforce and implement Good Hygienic Practices and HACCPS at all commercial meat retail outlets. For this process to be efficiently undertaken there is the need to improve staff strength and adequate logistics for effective monitoring.

Butchers should be given periodic training on food and meat safety through seminars, workshops and public education to update this knowledge on the adverse effects of poor personal and retail hygiene and sanitation.

Good hygienic practices (GHP) must be strictly adhered to by butchers and sellers, the equipment must be washed properly and periodically in a day

The Municipal Health Management Team (MHMT) should organize periodic retail and social health education at each Suburb, markets or worship centers to advocate for adherence to healthy life living through food and meat consumption.

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APPENDICES

APPENDIX 1

DIFINITION OF TERMS

Pathogen: any disease-producing agent, especially a virus, bacterium, or other microorganism.

Heavy Metals: any metal with a specific gravity of 5.0 or greater, especially one that is toxic to organisms, as lead, mercury, copper, and cadmium.

Microbe: a microorganism, especially a pathogenic bacterium.

Biochemical Test: a **test** commonly used when trying to identify Gram-negative enteric bacteria, all of which are glucose fermenters but only some of which produce gas.

Quality: character with respect to fineness, or grade of excellence.

Beef: the flesh of various bovine animals, especially the cow, when killed for eating **Hide:** an animal skin treated for human use. Hides include leather from cattle and other livestock animals, alligator skins, snake skins for shoes and fashion

Retail Outlet; a store that sells smaller quantities of products or services to the general public

Food borne illness: (also food borne disease and colloquially referred to as food poisoning) is any illness resulting from the consumption of contaminated food.

Contamination: the act of contaminating, or of making something impure or unsuitable by contact with something unclean, bad, etc.

Food Poisoning: an acute gastrointestinal condition characterized by such symptoms as headache, fever, chills, abdominal and muscular pain, nausea, diarrhea, and prostration, caused by foods that are naturally toxic, as poisonous mushrooms, by vegetable foods that are chemically contaminated, as by insecticides, or by bacteria or their toxins, especially of the genus Salmonella.

Total coliform*:* are a group of bacteria commonly found in the environment, the digestive systems of warm-blooded. Total coliform *counts* give a general indication of the sanitary condition of a water supply.

Total Viable Count (**TVC**): gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mold in a sample. To be specific, the count actually represents the number of colony forming units (cfu) per g (or per ml) of the sample.

Total Staphylococcus: any of several spherical bacteria of the genus *Staphylococcus*, occurring in pairs, tetrads, and irregular clusters. Some species such as *S. aureus*, can be pathogenic for humans.

Butcher: a person who slaughters certain animals, or who dresses the flesh of animals, fish, or poultry, for food or market.

Slaughter House: a building or place where animals are butchered for food; abattoir.

APPENDIX 2

Identifiable Characteristics of Gram Negative Bacteria Isolates in Retail Meats in Tarkwa Municipality

Characteristics	**Isolates**			
	1	2	3	4
Gram staining	-	-	-	-
Oxidase test	-	-	-	-
Mortility	-	+	+	-
Indole test	+	-	+	-
Catalase test	+	+	-	+
Methy red	-	+	+	+
Urease activity	-	-	+	+
Growth on KIA medium				
Slope	Y	Y	Y	Y
Butt	Y	Y	Y	Y
Hydrogen sulphide (H_2S)	-	+	-	-
Gas production	+	+	+	+
Sugar Fermentation tests:				
Glucose	A/G	A/G	A/G	A/G
Lactose	A	A/G	A	A
Sucrose	A	A/G	A	A
Manitol	A	A	A	A
Maltose	A	A/G	A	A
Most Probable Organism	**Ec**	**Ss**	**Es**	**Kp**

Keys:

Ec = Escherichia coli,, Ss = Salmonella, Es = Enterobacter spp, Kp = Klebsiella spp

A/G = Acid and gas production, - = No growth, + = Growth

A = Acid production only and no gas production, Y = Yellow (Acid reaction).

APPENDIX 3

Table 3: Microbial contamination characteristics of various meat sale environments stratified by Retailed outlet (*log of cfu/cm²*).

	Parameters	TVC	TCC	TSC
Beef	***Central Market***	5.03±1.02	3.83±0.93	2.97±0.59
	Layout	5.60±1.12	4.60±0.90	3.07±0.79
	p-value	0.7196	0.5754	0.9183
Knives	***Central Market***	2.42±0.81	1.42±0.32	0.78±0.15
	Layout	2.51±0.86	1.59±0.46	1.04±0.29
	p-value	0.9386	0.7734	0.4636
Wooden Boards	***Central Market***	2.04±0.80	1.79±0.72	1.26±0.45
	Layout	2.71±1.03	2.46±0.94	2.07±0.85
	p-value	0.6244	0.5915	0.4302
Weighing Scales	***Central Market***	0.94±0.19	0.76±0.17	0.49±0.02
	Layout	1.80±0.57	1.60±0.55	1.23±0.40
	p-value	0.2048	0.1969	0.1134
Shop Floor	***Central Market***	1.57±0.20	1.34±0.18	1.25±0.14
	Layout	2.44±0.36	2.35±0.36	2.05±0.33
	p-value	0.0773	0.0446	0.0667
Workers Hands	***Central Market***	1.84±0.70	1.59±0.68	1.40±0.59
	Layout	1.73±0.67	1.42±0.58	1.28±0.52

p-value	0.9172	0.8550	0.8843

Data is presented as means ± standard deviation. p is significant at < 0.05. . TVC- Total Viable Count, TCC- Total Coliform Count, TSC- Total Staphylococcal Count.

APPENDIX 4

Figure 4: A Meet Retail Kiosk (Outlet) at Karikwanaano Market

APPENDIX 5

Figure 5: Butchers at Slaughter yard in the Tarkwa Municipality