# INFLUENCE OF LACTIC ACID AND TRISODIUM PHOSPHATE TREATMENTS ON THE SHELF LIFE OF BEEF OBTAINED FROM RETAIL MARKETS IN ZARIA METROPOLIS, NIGERIA

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**AUGUST, 2016**

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**BY**

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**DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,**
**AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

**AUGUST, 2016**

## DECLARATION

I declare that the work reported in this dissertation, titled **'Influence of Lactic Acid and Trisodium Phosphate Treatments on the Shelf Life of Beef Obtained from Retail Markets in Zaria Metropolis, Nigeria'** was carried out by me in the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria – Nigeria. The information derived from literature have been duly acknowledged in the text and in the list of references provided. No part of this dissertation has been presented for another degree or diploma at this or any other institution.

Adaeze Juliet EZENWAFOR _____ _____

Signature Date

## CERTIFICATION

This thesis, entitled **'INFLUENCE OF LACTIC ACID AND TRISODIUM PHOSPHATE TREATMENTS ON THE SHELF LIFE OF BEEF OBTAINED FROM LOCAL RETAIL MARKETS IN ZARIA METROPOLIS, NIGERIA'** carried out by Adaeze Juliet EZENWAFOR meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

| | | |
|---|---|---|
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## DEDICATION

I dedicate this research work to the Almighty God and to my parents - my dad and my late mum, Sir John & Lady Blessing Ezenwafor.

## ACKNOWLEDGMENTS

Firstly, I render a deep and profound gratitude to God for His grace, love, strength and the enablement He gave to me in carrying out this work. This dissertation would not have been possible without the contributions of several people, to whom I am most grateful. I sincerely appreciate the Chairman of my supervisory Committee, Professor E.C. Okolocha, for his insight, support, encouragement and guidance in ensuring the best of this work. I also appreciate Dr M. Bello for his interest and guidance in bringing out the best of this research work.

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## ABSTRACT

Beef sold at retail markets can be contaminated with microorganisms which are of public health significance and impact on the shelf-life of meat. This study determined the influence of acid and alkaline decontamination interventions on the shelf-life of beef sold in retail markets in Zaria metropolis. Lactic acid (LA) and Trisodium phosphate (TSP) in two different volumes [1 liter (1L) and 2 liters (2L), respectively] were evaluated for their bactericidal effects on the bacterial population of beef sold in retail markets in Zaria. One hundred grams each of 216 beef samples were obtained from three retail markets: Samaru, Tudun-wada and Sabon gari; seventy-two samples (72) per market. Convenience sampling procedure was employed for sample collection. Twenty four out of 72 beef samples from each market, were sprayed on both sides for 10 seconds with LA (12 samples with 1L treatment and 12 with 2L treatment); a total of 24 meat samples were treated with TSP (12 samples with 1L treatment and 12 samples with 2L treatment) and another 24 samples with water (control) (12 with 1L and 12 with 2L). Sprayed samples were allowed to drip for 5mins and then maintained at 4$^{o}$C. Treated meat samples were evaluated for Aerobic Plate count (APC), Total Coliform count (TCC) and the presence of *E. coli* on days 0, 3 and 6 of storage. Two-way ANOVA was used to compare effect of various treatments on APC, TCC and isolation rate of *E. coli.* Generally, the mean log reduction values (CFU/ml) between days 0 and 6 for APC and TCC either increased or decreased for all the markets sampled. There was a significant difference recorded for the 1L ($p<0.05$) and 2L LA ($p<0.001$) treatment for Tudun-wada and Sabo markets, respectively as compared to the control giving 1.3 and 2.4 log cycle reductions respectively. TSP gave no significant reductions ($p>0.05$) from the control. Although, LA

treatment gave better reductions than the TSP treatment on the microbial load, other treatments with LA were not significantly different ($p>0.05$) from the control group. The effect of the decontaminants on the isolation rate of *E. coli* indicated that LA was effective against *E.coli* species, when compared to the control. Although, TSP treatment reduced *E. coli* consistently, the frequency of isolation was generally higher than what was obtained for the control and the LA-treated group on day 0 of storage. Beef samples treated with LA did not affect the aesthetic sensibilities of the consumer and a higher preference (57 %) was recorded for the treated roasted beef samples than the control (14 %) in terms of its appearance, smell and taste. Overall, the findings of this study revealed that the influence of LA and TSP treatment was not overly effective in prolonging the shelf-life of beef samples sold in retail markets in Zaria metropolis. Meat samples with LA treatments however showed lower isolation rate of *E. coli* thereby increasing the shelf-life. Good hygienic measures should therefore be employed during meat processing so as to increase its shelf-life and generally improve food safety.

Keywords: Lactic Acid, Trisodium Phosphate, Shelf-life, Beef, Retail markets

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## ABBREVIATIONS

| | |
|---|---|
| *et.al* | And Others |
| $^0$C | Degree Celsius |
| µm | Micrometer |
| mm | Millimeter |
| nm | Nanometer |
| % | Percentage |
| A.B.U | Ahmadu Bello University |
| ANOVA | Analysis of Variance |
| APC | Aerobic Plate Count |
| CFU | Coliform Forming Unit |
| DAEC | Diffusely Adherent *E. coli* |
| DFD | Dark, Firm and Dry |
| DNA | Deoxyribonucleic acid |
| EAEC | Enteroaggregative *E. coli* |
| EHEC | Enterohaemorrhagic *E. coli* |
| EIEC | Enteroinvasive *E. coli* |
| EPEC | Enteropathogenic *E. coli* |
| ETEC | Enterotoxigenic *E. coli* |
| FAO | Food and Agricultural Organisation |
| GDP | Gross Domestic Product |
| GRAS | Generally Regarded as Safe |
| HUS | Haemolytic Uremic Syndrome |
| IFST | Institute of Food Science and Technology |
| KGY | KiloGray |

| | |
|---|---|
| LA | Lactic Acid |
| LAB | Lactic Acid Bacteria |
| 1L | 1 Liter |
| 2L | 2 Liter |
| LOG | Logarithm |
| MRVP | Methyl Red Vogues Proskeur Broth |
| MetMB | Metmyoglobin |
| MAP | Modified Atmosphere Packaging |
| $MBO_2$ | Oxymyoglobin |
| PSE | Pale, Soft and Exudative |
| SIM | Sulphide Indole Motility |
| SPP | Species |
| SPSS | Statistical Package for Social Sciences |
| TCC | Total Coliform Count |
| TSI | Triple Sugar Iron |
| TSP | Trisodium Phosphate |
| TVC | Total Viable Count |
| UV | UltraViolet |
| USDA | United States Department of Agriculture |
| USFDA | United States Food and Drug Administration |
| UTI | Urinary Tract Infection |
| aW | Water Activity |
| WT/VOL | Weight/Volume |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of the Research Study

Meat is a nutritious and protein-rich food, which is highly perishable. It has a short shelf-life when good preservation methods are not used (Olaoye and Onilude, 2010). It is an important source of fats, vitamins and essential minerals. It is a high quality protein source that contains almost all the essential amino acids required in the human body (Lafarga and Hayes, 2014; Klurfeld, 2015), making it suitable for use as food. Meat, though considered being highly desirable and nutritious, is also highly perishable. This is because it provides the nutrients needed to support the growth of many types of microorganisms (Buncic *et al*., 2014). Due to its unique biological and chemical nature, meat undergoes progressive deterioration from the time of slaughter of an animal until consumption.

Spoilage of meat has remained a serious challenge in developing countries, including Nigeria, for decades (Olaoye and Ntuen, 2011). This has been due to poor storage systems in such countries where necessary facilities that could help promote preservation are unavailable. Where available, unsteady power supply necessary to maintain such facilities has however constituted a serious problem, thereby rendering them to function below their maximum capacity. Furthermore, the ambient temperature in developing countries that are in tropical regions is usually about 30 $^{o}$C or above. Meanwhile, most spoilage organisms have been found to have their optimum growth temperature within such range (30 $^{O}$C – 45 $^{O}$C) (Faid and Ahami, 2004; Olaoye and Ntuen, 2011). Meat surfaces are also known to contain several microorganisms (Bibek, 2005; Ireneous, 2008), some of which are reported to cause zoonotic diseases in humans (Norrung and Buncic, 2008). Spoilage by microbial growth is the most important factor in

relation to the keeping quality of meat (Tajkarimi *et al*., 2010). Meat can also be a source of resistant bacteria, which could potentially be spread to the community through the food chain (Gousia *et al*., 2011), making decontamination imperative.

Decontamination of meat carcasses is primarily aimed at the elimination of microorganisms. These organisms inevitably contaminate meat carcasses during dressing operations without irreversibly deteriorating the sensory quality. The disinfection of fresh warm carcasses prevents the spread of pathogens from contaminated meat carcasses to other sound meat carcasses in the chilling rooms, the meat environment and the initial sterile muscle meat during butchering operations. Acid or alkaline decontamination of meat carcasses exploits principally an immediate bactericidal activity, whose lactate anion slows down the growth of surviving microbes during storage (Dincer and Baysal, 2004; Siragusa, 1995). Organic acids have been of considerable value as food preservatives. They are also food ingredients and often naturally produced by microorganisms (Theron and Lues, 2007). Use of organic acids reduces bacterial counts in the meat surface layer. Lactic acid is often used because it is a natural meat compound produced during post-mortem glycolysis. Trisodium phosphate (TSP) is a highly alkaline compound which is usually applied as dip or spray of pre-chilled or chilled poultry carcasses (Capita *et al*., 2002; Oyarzabal, 2005). The alkalinity of TSP causes bacterial death through disruption of cell membrane (Mendonca *et al*., 1994; Sampathkumar and Khachatourians, 2003). It acts as a detergent that removes fat from the surface of poultry carcasses, consequently, increasing its killing action (Kim *et al*., 1994). Trisodium phosphate is an acceptable decontaminant because it does not affect the sensory quality (smell, colour, flavor, texture and overall acceptability) of carcass treated with its solutions, even as high as 8 and 10 % (Capita *et al*, 2000; Okolocha and Ellerbroek, 2005).

Therefore, since there are no indications that these chemical decontaminants could in any sense endanger human health, there is a need for effective reduction of the number of contaminating microorganisms of public health significance on the surface of meat carcasses. An effective decontamination operation could also anticipate increasingly stringent demands for improved meat hygiene.

## 1.2 Statement of Research Problem

Contaminated raw meat is one of the major sources of food-borne illness (Bhandare *et al*., 2007; Podpecan *et al*., 2007). Poor meat handling, transportation and sales practices subject meat to contamination leading to poor quality and exposure of human consumers to health risks (Adeyemo *et al*., 2009). The levels of contamination in traditional/retail meat shops are significantly higher than found in abattoir (Bhandare *et al*., 2007). In most developing countries including Nigeria, the absence of the existing hygienic practices in slaughtering, transportation and marketing has been found to be one of the major causes of meat contamination by pathogenic and non-pathogenic microorganisms (FAO, 2004). In Zaria, high microbial load have been seen for meat purchased at retail markets for minimum counts of the microbial load exceeding even the allowed permissible value having counts as high as 6.0 $\log_{10}$ and 4.7 $\log_{10}$ for the Aerobic Plate Counts and Total Coliform Counts respectively (Lawan *et al*., 2011). This microbial surface contamination has been repeatedly reported to have a significant effect on the meat shelf-life. Moreover, healthy-food producing animals can be carriers of important bacterial pathogens causing human illness (EFSA/ECDC, 2014). Such pathogens might enter the food chain by direct or indirect faecal contamination if good hygiene practices are not warranted thereby spreading such diseases as salmonellosis, cholera, *E. coli* food poisoning and listeriosis,

caused by meat contamination. This constitutes a serious public health concern (Neil *et al*., 2002; Zweifel *et al*., 2014).

## 1.3 Justification

Microbial food safety is also of increasing public health concern worldwide. Raw beef sold at retail outlets in Nigeria undergo a considerable amount of handling and contacts with microbes of different sources (Umoh, 2002). Unhygienic processing of carcasses is a common practice in Nigeria, resulting in carcass contamination and isolation of microorganisms from meat and slaughtering facilities (Umolu *et al*, 2006; Ojo *et al*, 2009). Bacterial organisms such as *E. coli* increase or decrease during processing depending on the level of faecal contamination of live cattle, efficiency of evisceration and hygienic practices in the abattoir (Rigobelo *et al*., 2006). Due to the poor environmental conditions of abattoirs and slaughter slabs, there is a very high risk of contamination of these carcasses during preparation (James *et al*., 2000). By reducing the primal surface contamination and limiting the microbial growth, the shelf-life of beef can be considerably prolonged, as well as, improve food safety (James *et al*., 2000).

The meat retail market is one of the supply chains of the beef industry. It is a critical and very vital point of the supply chain because meat are retailed to consumers at affordable prices. It is the central point where most consumers purchase their meat. In Nigeria, fresh beef forms a significant proportion of meat intake (Olaoye and Onilude, 2010). It is either eaten cooked or processed into other forms to avoid associated spoilage. Means of controlling or even improving the safety of food products is to decontaminate the meat during or at the end of the production line. The decontamination of beef can help to reduce human food-borne infection (Dincer and Baysal, 2004). It can also help to prolong its shelf-life and enhance its keeping quality in situations where refrigeration is poor due to unstable power supply as observed in Nigeria. There

is paucity of information on the control of pathogenic organisms associated with fresh beef obtained from retail markets in Zaria as regards decontamination. Hence, investigations are required in order to extend meat shelf-life and promote the health and safety of consumers of fresh beef.

## 1.4 Aim of the Study

The aim of the study was to determine the influence of lactic acid and trisodium phosphate treatments on the shelf-life of beef obtained from retail markets in Zaria metropolis, Nigeria.

## 1.5 Objectives of the Study

The specific objectives of the study were to determine the:

a. Effect of lactic acid and trisodium phosphate on the microbial quality of beef bought from retail markets in Zaria metropolis.
b. Effect of lactic acid and trisodium phosphate on *E. coli* contaminated beef sold in retail markets in Zaria.
c. Acceptability or otherwise of the treated beef samples using sensory analyses.

## 1.6 Research Questions

a. What is the effect of trisodium phosphate on the microbial load of beef sold in retail markets in Zaria metropolis?
b. What is the effect of lactic acid treatment on the microbial load of beef sold in retail markets in Zaria metropolis?
c. Are treatments with trisodium phosphate effective against *E. coli* contamination on beef sold in the retail markets in Zaria metropolis?
d. Are treatments with lactic acid effective against *E. coli* contamination on beef sold in the retail markets in Zaria metropolis?

e. Do the treatments with trisodium phosphate have effect on the sensory qualities of beef sold in retail markets in Zaria metropolis?

f. Do the treatments with lactic acid have effect on the sensory qualities of beef sold in retail markets in Zaria metropolis?

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Beef and its Significance

Meat is animal flesh that is eaten as food (Lawrie and Ledward, 2006). It is a rich source of nutrient for human nutrition. It is an important source of essential amino acids, vitamins, minerals and also long chain poly-unsaturated fatty acids (Salobir, 2000). Meat is a part of the

human diet in most cultures and can be prepared as steaks, in stews, as dried meat, in sandwiches, in canned foods, fried or roasted. Beef is the culinary name for meat from bovines, especially cattle. It is the third most widely consumed meat in the world, accounting for about 25% of meat production world-wide, after pork and poultry at 38% and 30%, respectively (Ravloff, 2003). It is an excellent source of protein (51%), zinc (38%), vitamin B12 (37%), selenium (26%), phosphorous (20%). It is also a good source of niacin (17%), vitamin B6 (15%), iron (14%) and riboflavin (12%) (USDA, 2007). High quality beef proteins, alongside iron and zinc, promote optimal health and provide consumers with a naturally nutrient-rich package (Klurfeld, 2015). It also brings satisfaction and nutrition from their calories without sacrificing taste

Beef cattle production is a big animal industry within Nigeria and throughout the world (Benard *et al.*, 2010). Rumination allows cattle to digest fibrous feeds that cannot be directly consumed by humans and thus to make a net positive contribution to food balances (Gerber *et al*., 2015). Other than its consumption, other significance of beef cattle includes by-products, which are beneficial and serve mankind. They include leather goods, fertilizers, cosmetics, drugs, hair products, perfumes, gelatin products, glues and animal feed by-products. Economically, it is an important area of activity in Nigeria contributing about 5% of GDP in the Nigerian economy (Bernard *et al*., 2010). Beef cattle is produced and marketed in all parts of Nigeria but with the North-East and North-Western part predominating in cattle production (Mamman, 2005). It also provides employment or jobs such as stock breeders, buyers, market intermediaries, cross border handlers, truck hauliers, herders, butchers and retailers. These play their part in improving the Nigerian economy and serving its populace in the area of job creation, health and nutrition.

## 2.2 Contamination of Beef

Food-borne pathogens are the leading cause of illness and death in developing countries, costing billions of dollars in medical care and social costs (Fratamico *et al.,* 2005). Raw meat generally refers to any type of uncooked muscle tissue of an animal used for food (Mboto *et al.*, 2012). It is an ideal medium for bacteria growth due to its high moisture content, high protein constituents, fermentable carbohydrate, favorable pH and other growth factors (Buncic *et al.,* 2014). This makes contaminated raw meat one of the main sources of food-borne illness (Bhandare *et al.*, 2007; Podpecan *et al.*, 2007). These favorable chemical compositions, which it possesses, expose it to the contamination by spoilage and pathogenic bacteria when adequate hygienic measures during the preparation, transport and marketing are not respected (Hudson *et al*, 2006).

Microbial contamination of meat starts during processing on the slaughter line (Pipek *et al.*, 2004). The hygienic adequacy of slaughter processes and the hygiene status of carcasses are increasingly under review (Adeyemo *et al.*, 2009, Adzitey *et al*., 2011, Makita *et al*., 2011). Poor waste management at slaughter houses also poses a great risk to meat contamination. A frequent isolation of *E.coli*, *Bacillus* spp, *Staphylococcus* spp, *Streptococcus* spp, *Salmonella* spp *and Campylobacter* spp from rumen/stomach wastes attests to this fact (Nwanta *et al.*, 2010). In addition, some of these organisms have been shown to develop antimicrobial resistances which are of public health significance from the viewpoint of food hygiene (Kabir *et al*., 2002; Stevenson *et al.*, 2006). This could be as a result of undue slaughtering of animals which have been treated with antimicrobial drugs in an abattoir or its environs. The wastes of these animals have been seen to contain antimicrobial substances, resulting in the antimicrobial resistance of the microorganisms present in the abattoir at such time. Kabir *et al*. (2002) observed the presence of thirty-seven (7.4%) positive urine samples containing antimicrobial substances from 500 cattle

slaughtered routinely in Zaria. This poses great public health significance from the viewpoint of food hygiene.

Meat transportation and handling from slaughter houses to retail markets are usually done in a most unhygienic manner (Bello *et al.*, 2015; Fearon *et al.*, 2013; Niyonzima *et al.*, 2013). In many developing countries including Nigeria, meat is normally transported to markets either in vans, motorcycles and at times, even using bicycles. In most instances, the transportation systems are made up of surfaces that are difficult to clean and disinfect (Adzitey *et al.*, 2011). In most developing countries, the absence or non-respect of the existing hygienic practices in slaughtering, transportation and marketing has been found to be one of the major causes of meat contamination by pathogenic and non-pathogenic microorganisms (FAO, 2004). Poor meat handling, transportation and sales practices subject meat to contamination leading to poor quality and exposure of human consumers to health risks (Adeyemo *et al.*, 2009). Microbes such as *Streptococcus* spp, *Staphylococcus* spp, *Enterococcus* spp, *Salmonella* spp and *E.coli, Bacillus spp, Proteus spp, Pseudomonas spp, Aspergillus spp, Penicillium spp and yeast* have been isolated from meat samples (Adzitey *et al.*, 2010; Eze and Ivuoma, 2012). It has been shown that the level of contamination in traditional/ retail meat shops are significantly higher than found in the abattoir even though microbial contamination in the abattoir is also high when compared to reports from developed countries (Bhandare *et al.*, 2007). These results show that meat carcasses are often contaminated with microorganisms due to poor handling and transportation as well as unhygienic and poor sanitary conditions.

Contamination of animal carcasses and raw meat by micro-organisms could also be as a result of exposure to abusive conditions of handling and storage that allow microbial proliferation. This increases the potential for presence of pathogenic bacteria and formation of toxins. Cattle feces

and hides contribute to meat contamination with *Escherichia coli* O157:H7, ultimately impacting beef safety (Jacob *et al*., 2010). Beef contamination is a direct result of pathogen transfer from cattle hides harboring organisms such as enterohemorrhagic *Escherichia coli.* Hide contamination occurs from direct and indirect fecal contamination in cattle production and lairage environments (Arthur *et al*., 2010). Other sources contributing microbial contamination to animal carcasses and to fresh meat during slaughter and dressing include soil, water, air, intestines, lymph nodes, processing equipment, utensils and humans ( Zhao, 2015).

Within the beef slaughter and dressing process, holding pens, carcass skinning and evisceration have been identified as probable introduction points of major contamination (Anon, 1993). Niyonzima *et al*., (2013) reported that between four stages of carcass preparation (skinning, dressing, transport and marketing), the total aerobic count between dressing, transportation and marketing stages increased. In the same study, it was also noted that in-between the skinning and marketing stages, there was a 5.8 log cfu increase in the total aerobic bacterial counts indicating that a lot of contamination occurs during meat slaughtering, transportation and marketing (Niyonzima *et al*, 2013).During bleeding, skinning and cutting, the main sources of microorganisms are the exterior of the animals which includes the hides, hooves and hair (Norrung and Buncinc, 2008). Hand and knife swabs were also collated for the same study and it was found out that unrinsed operatives' hands and knives also introduced contamination comparable to hide-carcass contact for those operations in which hide-hand contact is unavoidable.

It is therefore worth considering that elimination of pathogens at the pre-harvest level is unlikely. The contamination of animal carcasses and raw meat by microorganisms, including spoilage and pathogenic types, is practically unavoidable (Koutsoumanis and Sofos, 2004; Koutsoumanis *et*

*al*., 2005). The exterior of the animal harbors large numbers and many kinds of microorganisms from the soil, wash water, feed and manure as well as its natural surface flora and intestinal contents containing the intestinal organisms. In addition, knives, clothes, air, environment of the slaughter slabs/abattoir, hands and clothing of the workers and the physical facilities can serve as intermediate sources of contaminants.

The presence of these microbial contaminants is of major concern because it can cause adverse effects on human health and compromise food safety (Bacon and Sofos, 2003; Sofos, 2004). In efforts to meet regulatory requirements and ultimately provide safe products to consumers, a variety of interventions with the objective of reducing contamination on carcass and meat products should be taken. Meat handlers/processors should observe strict hygienic measures so that they may not serve as source of chance inoculation of microorganisms and faecal contamination of fresh meat and other meat products. Decontamination processes to reduce or eliminate the microbial load on meat carcasses should be employed. Cooking processes and hygiene will greatly reduce the microbial load to a harmless level. Thorough cooking as well as good hygiene in order to prevent contamination of food eaten raw is also important (Manios *et al*., 2015). Sanitary authorities should ensure and enforce strict compliance to the recommended food standards as regards to the processing and sales of fresh and packaged meat products (Tomasevic *et al*., 2016). Also, meat handlers and sellers should be educated on the adverse effect of lack of proper personal hygiene and sanitation. In addition, good manufacturing practices should be adhered to by butchers and those selling meat (Petruzelli *et al*., 2016). Veterinary doctors should inspect the animals to be slaughtered before the meat is sold to the general public (Eze and Ivuoma, 2012).

## 2.3 Organisms Associated with Spoilage of Meat

Microorganisms are living entities of microscopic size and include bacteria, viruses, yeasts and moulds (together designated as fungi), algae, and protozoa (Ray and Bhunnia, 2007). Except for a few sterile foods, all foods harbor one or more types of microorganisms. Some of them have desirable roles in food, such as in the production of naturally fermented food, whereas others cause food spoilage and foodborne diseases in foodstuffs and other perishable goods. Meat is a foodstuff that can be spoiled extremely quickly. It is a complex niche that has chemical and physical properties which allow the colonization and development of a variety of organisms. According to Bruckner *et al.* (2012), microbial meat spoilage is influenced by a large number of factors which can be divided into: intrinsic, extrinsic, processing and implicit factors. The intrinsic factors comprise the physical and chemical properties of the food stuff such as water activity, nutrients, structure of foodstuff. Extrinsic factors comprise the storage condition such as the storage temperature and composition of atmosphere. Processing factors are physical or chemical methods of treating foodstuffs during processing, like cooking. Intrinsic factors are a reflection of the synergistic or antagonistic effects between bacteria.

The contamination of carcasses usually occurs during the conversion of living animals into meat for human consumption (Bornadi *et al*., 2013 ; Khen *et al*., 2014). Generally, tissues under the hide of healthy cattle are usually sterile (Anderson *et al*., 1992) and contamination of meat surfaces with microorganisms begins at slaughter, then continues throughout the butchering and beyond (Baxter, 2000). These slaughtering and butchering of food animals provide bacteria with an opportunity to colonise meat surfaces. A wide range of microorganisms coming from different sources are introduced to surfaces which contain abundant nutrients and have high water availability (Nychas *et al*., 2008). Their numbers soon reach levels that cause sensory deviations and lead finally to spoilage of meat (Doulgeraki *et al*., 2012). The survival and growth of these

microbes is influenced, to a great extent, by the composition of the atmosphere and environment surrounding the meat. This environment then enforces a selection pressure on the bacterial community, and those groups of bacteria best adapted to the environment will outgrow the others, become dominant, and reach high numbers. Thus, the survival, growth and succession of specific spoilage bacteria can be affected by a diversity of ecophysiological factors in the physical and chemical environment. These factors, including meat constituents, temperature, pH, oxygen or carbon dioxide (packaging atmosphere) and competing microbiota, are important in maintaining meat quality over time (Koutsoumanis *et al*., 2006; Lambert *et al*., 1991).

Proteinaceous foods are vulnerable to the activities of microorganisms because of their nutrient and water content, and slightly acidic pH (Hius in't Veld, 1996). The microflora that develops on fresh meats will depend on the method of packaging that is used for the product. Fresh meat can either be packaged in: air, where the meat is wrapped in a gas permeable plastic; vacuum, where all the air is removed from within the package so that the gas-impermeable packaging material forms a skin around the meat and minimizes the transmission of oxygen (Seidmand and Durland,1982); or modified atmosphere packaging (MAP), where the air within the package is removed and generally replaced with carbon dioxide and air or oxygen or nitrogen in various combinations (Baxter, 2000). Pennacchia *et al.* (2011) in a study to ascertain the spoilage-related microbiota associated with chilled beef stored in air or vacuum pack found out that vacuum packaging reduced the viable counts of *Brochothrix thermosphacta, Pseudomonas* spp. and *Enterobacteriaceae* than those stored in air, whereas the growth of lactic acid bacteria (LAB) was unaffected. This study reveals that the spoilage of raw meat is mainly due to undesired microbial development in meat during storage.

When large numbers of microorganisms are present in raw meat, there will be changes such that it becomes unappealing and unsuitable for human consumption (Fung, 2010; Gram *et al*, 2002). The type of bacteria and their microbial load depends on the initial meat contamination and on the specific storage conditions that can influence the development of different spoilage-related microbial populations thus affecting the type and rate of spoilage process (Doulgeraki *et al*., 2012). This initial microbial load of meat depends on the physiological status of the animal at slaughter, the spread of contamination at slaughter houses and during processing (Nychas *et al.*, 2008). The most frequent bacteria genera to occur during chilled storage of raw meat in different conditions are as follows: *Aeromonas* spp, *Acinectobacter* spp, *Achromobacter* spp, *Alcalgenes* spp, *Alteromonas* spp, *Arthrobacter* spp, *Bacillus* spp, *Bradyrhizobium* spp, *Brochothrix thermosphacta, Buttiauxella* spp, *Carnobacterium* spp, *Chromobacterium* spp, *Citrobacter* spp, *Clostridium* spp, *Enterobacter* spp, *Escherichia coli, Hafnia* spp, *Klebsiella* spp, *Kluyvera* spp, *Koruria* spp, *Kurthia* spp, *Lactococcus* spp, *Lactobacillus* spp, *Leuconostoc* spp, *Limnobacter* spp, *Listeria* spp, *Microbacterium* spp, *Micrococcus* spp, *Moraxella* spp, *Paenibacillus* spp, *Pantoea* spp, *Photobacterium* spp, *Proteus* spp, *Providencia* spp, *Pseudomonas* spp, *Psychrobacter* spp, *Rahnella* spp, *Ralstonia* spp, *Rudaea* spp, *Serratia* spp, *Shewanella* spp, *Staphylococcus* spp, *Stenotrophomonas* spp, *Streptococcus* spp, *Weisella* spp, *Yersinia* spp (Doulgeraki *et al*, 2012).

Basically, two bacteria groups cause meat spoilage, namely: Gram-positive and Gram-negative bacteria. Varying levels of both Gram-positive and Gram-negative bacteria constitute the initial microbial population. Adaptation and resistance to conditions in and around the meat surface will determine which group among the initial contaminants will eventually survive (Holzaffel, 1998). Gram-negative bacteria are a group of bacteria that do not retain the crystal violet stain used in

the gram staining method of bacterial differentiation, making positive identification possible (Baron *et al*, 1996). They constitute the greatest spoilage potential for meat and meat products (Garcia-Lopez, 1998). Gram-negative bacterial foodborne pathogens are a worldwide cause of morbidity and mortality (Mor-Mur and Yuste, 2010). They are the bacteria which develop on carcass meats stored in air under refrigeration conditions and are the principal group in aerobic spoilage (Baxter, 2000; Sentence, 1991). Members of the genera *Pseudomonas, Acinetobacter, Psychrobacter, Moraxella, Shewanella, Enterobacteriaceae* are examples. Gram-positive bacteria are bacteria that give a positive result in the gram stain test. They take up the crystal violet stain used in the test, and then appear to be purple-coloured when seen through a microscope. According to Holzaffel (1998), the Gram-positive bacteria that constitute the initial microbial population are represented by *Micrococci* followed by lactic acid bacteria and *Brochothrix thermosphacta.* This confirms the study carried out by Borch *et al.* (1996), who when conducting a study on the bacterial spoilage of meat and cured meat products for beef and pork found that the predominant Gram-positive bacteria associated with spoilage of refrigerated beef and pork are *Brochothrix thermosphacta*, *Carnobacterium* spp, *Lactobacillus* spp, *Leuconostoc* spp. In more recent studies, Pennachia *et al.* (2011) stated that meat spoilage is most frequently caused by the following groups of bacteria: *Pseudomonas* spp, *Enterobacteriaceae, Brochothrix thermosphacta*, lactic acid bacteria.

Considering the present study, *E. coli* from the member of the *Enterobacteriaceae* family will be considered. Generally, the *Enterobacteriaceae* family is a large family of Gram-negative bacteria that are rod-shaped, and are typically 1-5um in length. They are facultative anaerobes, fermenting sugars to produce lactic acid and other end products. Majority are motile and they are generally non-spore forming. They are classified in the order Enterobacteriales of the class

Gammaproteobacteria and grouped in the phylum Proteobacteria (Brenner *et al*., 2005). They include *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella, Shigella, Proteus, Enterobacter, Serratia and Citrobacter*. *E. coli* is a Gram-negative, facultatively anaerobic, catalase positive, oxidase negative, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. *E. coli* has also been widely exploited as a cloning host in recombinant DNA technology. It can also be a highly versatile, and frequently deadly pathogen. Several different *E. coli* strains cause diverse intestinal and extra intestinal diseases by means of virulence factors that affect a wide range of cellular processes (Kaper *et al*., 2004). They are characterized by shared O (lipopolysaccharides) and H (flagellar) antigen that define serogroups (O antigen) and serotypes (O and H antigen) (Nataro and Kaper, 1998).

Among the intestinal pathogens, there are six well described categories: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* ( ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC).

EPEC are foodborne pathogens that cause potentially fatal infant diarrhea especially in developing countries (Alonso *et al*., 2012). EPEC strains are defined by the characteristic attachment and effect they elicit upon interaction with epithelial cells, and the fact that they do not produce Shiga toxins (Kaper, 1996). Symptoms include watery diarrhea sometimes accompanied by low-grade fever and vomiting. EHEC causes bloody diarrhea (haemorrhagic colitis), non-bloody diarrhoea and haemolytic uremic syndrome (HUS) (EFSA, 2011). The EHEC strain most commonly found is O157: H7 (Ojo *et al*., 2010). They produce verotoxin (so-called because of its ability to kill Vero- African green monkey kidney cells. ETEC causes watery diarrhea, which can range from mild, self-limiting disease to severe purging disease. The

organism is an important cause of childhood diarrhea in the developing world and is the main cause of diarrhea in travelers to developing countries (Nataro and Kaper, 1998). It colonizes the surface of the small bowel mucosa and elaborates enterotoxins, which give rise to intestinal secretion. Diarrhea caused by ETEC has a lot in common with cholera expressed by *Vibrio cholerae* (Spangler, 1992). EAEC are increasingly recognized as a cause of often persistent diarrhea in children and adults in both developing and developed countries, and have been identified as the cause of several outbreaks worldwide (Kaper *et al*., 2004). They possess an enterotoxin known as *Shigella* enterotoxin 1 owing to its presence in most strains of *Shigella flexneri* 2a (Henderson *et al*., 1999). EIEC are biochemically, genetically and pathogenically closely related to *Shigella* spp. Numerous studies have shown that *Shigella* and *E. coli* are taxonomically indistinguishable at the species level (Wei *et al*., 2003). EIEC invades and multiplies within the epithelial cell of colon causing ulceration and inflammation but their strains do not produce Shiga toxin. DAEC have been implicated as a cause of diarrhea in several studies, particularly in children above 12 months of age (Scaletsky *et al*., 2002). Other extra intestinal diseases caused by *E. coli* include urinary tract infections (UTIs), meningitis and sepsis.

## 2.4 Bacterial Attachment to Meat Surfaces

### 2.4.1 Public health significance of bacterial attachment

Meat surfaces are contaminated with bacteria during slaughter and processing (Zulfakar *et al*. 2012). These various bacteria cause microbial spoilage of meat and it is influenced, not only by their initial attachment to the surface, but also by their subsequent proliferation after attachment (Chung *et al*., 1989). The attachment of these potential spoilage and pathogenic bacteria to food contact surfaces and the subsequent biofilm formation represent serious challenges to the meat

industry, since these may lead to cross-contamination of the products, resulting in lowered shelf-life and transmission of diseases (Giaouris *et al*., 2014). This buttresses the fact that bacterial transfer events are considered to be an important cause of transmission of food-borne diseases (Perez-Rodriguez *et al*., 2008).

Microbial attachment to surfaces is also of significance for food safety and spoilage, and the ability of microorganisms to adhere to surfaces has significant implication for food science and the medical industry (Palmer *et al*., 2007). For instance, Silagyia *et al*. (2009) noted that biofilm formation by *E. coli* O157:H7 on food contact surfaces poses a grave concern for the efficient control of the pathogen particularly in products that require no heating or cooking prior to consumption. Finished product contamination during beef processing could occur (Arthur *et al*., 2014). These pathogens originating with raw products can attach to food preparation surfaces, which if not adequately cleaned before re-use, can serve to recontaminate cooked foods (Frank, 2001). Understanding bacterial attachment properties to specific structures of meat could result in more targeted interventions to improve its safety and quality (Zulfakar *et al*., 2012).

### 2.4.2 Mechanism of bacterial attachment

Bacteria have a strong tendency to attach to surfaces. Attached cells will form a colony (biofilm) consisting of prokaryotic cells, surrounded by a matrix of biomolecules secreted by the cells (Boland *et al.*, 2000). The ability to attach to and subsequently detach from surfaces is a characteristic of microorganisms (Palmer *et al*., 2007). Attachment is advantageous and perhaps necessary for survival in the natural environment and offer protection from environmental stresses. Microbial attachment is a complex phenomenon and also the initial event in microbial infections which is influenced by the cell surface, the attachment surface, and the surrounding

medium (Frank, 2001). The mechanism of attachment of bacteria to surfaces generally involves a two-stage process: initial reversible attachment and subsequent irreversible attachment. This idea has been accepted by a majority of researchers (Frank, 2001; Garrett *et al.*, 2008; Selgas *et al.*, 1993). The term 'reversible' and 'irreversible' also corresponds to the terms 'loosely' and 'strongly' attached bacteria used by Firstenberg-Eden *et al*. (1978).

Reversible Attachment: Microorganisms approach the contact surface of the host or substratum, either by flagella that help in bacterial motility or by being transported to the surface through external physical forces such as fluid flow (Frank, 2001; Garrett *et al.*, 2008). Generally, pathogenic bacteria and adhesive substratum on most biological and inert surfaces are negatively charged (Frank, 2001; Ofek *et al.*, 2003), repelling bacteria from the surface. In this situation, there is an overall tendency for mutual repulsion by the two surfaces. The forces that allow the bacterial cell to overcome these repulsive barriers include van der Waals interactions, coulombic forces, hydrophobic interactions and eventually complementary interactions (Frank, 2001; Shoaf-sweeney and Hutkins, 2009). As the bacterial cell approaches closer to the surface of the substratum (10-100 nm), maximum repulsion occurs in that region between the overlapping electrical double layer due to the net negative charges of the opposing surface (Garrett *et al.*, 2008). Bacterial cell appendages facilitate the close contact of the cell and the surface by reducing overall electrostatic repulsion (Fletcher, 1980; Ofek *et al*., 2003). These bacterial physical appendages include flagella, fimbriae and pili. At a closer distance, hydrophobic interactions mediated by the hydrophobic moieties on the bacteria can be removed by shear forces associated with the washing process and turbulent flow of the surrounding fluid (Bayoudh *et al.*, 2006).

Irreversible Attachment: This is the second stage of bacterial attachment to a host or a substratum. It is initiated when the bacterial appendages acts as a bridge to reduce the distance between the cell and the substratum (Weiner *et al*., 1995). In time, extracellular binding polymers are then produced by the bacteria on the surface, thereby effectively anchoring the bacteria to the surface forming stronger permanent bonds with the surfaces (Allison and Sutherland, 1987; Oliveria, 1992). These structures may pre-exist on the bacterial cell surface before attachment occurs or their production is induced following attachment (Fletcher and Floodgate, 1973; Ofek *et al.*, 2003). Irreversible attachment of bacteria to meat tissues may also occur via physical entrapment of cells with the muscle fibres making it difficult to remove them (Frank, 2001).

### 2.4.3 Attachment of bacteria to meat surfaces

Attachment of bacteria to meat surfaces is influenced by various factors which include hydrophobicity and cell surface charge (i.e. overall net charge on a specific surface), bacterial structures, type of meat surfaces, pH, temperature, cell concentration, presence of chemical substances (Selgas *et al.*, 1993). Attachment of bacteria to the meat surfaces occurs rapidly in the first minute of contact between bacterial suspensions and meat tissue (Butler *et al*., 1979). These observations indicate that initial attachment is due to physiochemical properties of bacteria and the contact surface.

Before they can attach, microorganisms must reach the beef surface, either by their own power (motility) or by being transported (Frank, 2001). Bacterial adhesion on meat tissue not removed by thorough rinsing are often considered attached.

Muscle fibres undergo radial shrinkage and pull away from the surrounding endomysia when rigor develops. The resulting gaps provide a route for bacterial penetration (Gill *et al*., 1984). Cell motility and proteolysis enhance penetration especially in the presence of excess water which may enlarge these gaps. Gill *et al*. (1984) concluded that the fluid in gaps between muscle fibres in chicken meat presents little barrier to the passage of motile bacteria. The presence of proteolytic bacteria could speed up the penetration of non-proteolytic types (Gill and Penny, 1977, 1982). Proteolysis aids penetration but it is not a requirement (Thomas *et al*, 1987b). The ability of bacteria to penetrate meat tissue has significant implications for the effectiveness of a decontamination process, as these cells will be protected from the effect of sanitizing chemicals. Woody *et al*. (2000) showed high pH treatment were ineffective in killing *E. coli* O157:H7 cells, which have penetrated deep into beef muscle tissue. Therefore, this indicates that deep penetration reduces the effectiveness of decontamination procedures (Narendran, 2003).

## 2.5 Shelf-life of Beef

### 2.5.1 Meat spoilage

Meat is a nutritious, protein-rich food which is highly perishable and has a short shelf-life unless preservation methods are used (Addis, 2015). Generally, spoilage is said to be a state of a particular food in which it is offensive to consumers' senses. It is usually caused by metabolites of contaminant microorganisms (Paulsen and Smoulders, 2003). According to Nychas *et al*. (2008), spoilage is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations, and background, as well as, by the sensory acuity of the individual and the intensity of the change. Meat is a substance very much susceptible to spoilage due to chemical and enzymatic activities. The breakdown of fats, protein and carbohydrates of meat

results in the development of gross discoloration, strong off-odours, off-flavours and the development of slime which make the meat objectionable for human consumption (Dave and Ghaly, 2011). Growth of bacteria and spoilage of meat depends on the species of bacteria, nutrient availability, pH, temperature, moisture and gaseous atmosphere (Cerveny *et al.*, 2009).

There are three main mechanisms for meat and meat product spoilage after slaughtering, during processing and storage. They are namely: microbial spoilage, lipid oxidation and autolytic enzymatic spoilage.

a. Microbial spoilage of meat is said to constitute a significant portion in regards to meat loss and degradation (Heinz and Haut zinger, 2007). It is a factor that depends upon the condition of the carcass at the time of slaughter, the type of packaging, as well as, storage conditions of the meat (Dave and Ghaly, 2011). Meat provides an excellent growth media for a variety of microflora (bacteria, yeasts and moulds), some of which are pathogens (Jay *et al.*, 2005). The intestinal tracts and the skin of the animal are the main sources of these microorganisms. The microbiological quality of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, temperature and other conditions of storage and distribution (Olaoye and Ntuen, 2011).

b. Lipid oxidation is one of the primary mechanisms of quality deterioration in foods especially in meat products. The changes in quality are manifested by adverse changes in flavor, color, texture and nutritive value (Ripoll *et al.*, 2013), and the possible production of toxic compounds. Lipid oxidation in muscle systems is initiated at the membrane level in the intracellular phospholipid fractions (Gray *et al.*, 1996). It is the reaction of oxygen

with double bonds of fatty acids (Hutlin, 1994). After slaughtering of animals, the fatty acids in tissues undergo oxidation when the blood circulation stops and metabolic processes are blocked (Gray and Pearson, 1994; Linares *et al*., 2007).

c. Autolytic enzymatic spoilage is a natural process in the muscle cells of the animals after they have been slaughtered (Dave and Ghaly, 2011). The enzymes have the ability to combine with other organic compounds and work as catalysts for chemical reactions that finally end up in meat self-deterioration (Tauro *et al*., 1986). Here, carbohydrates, fats and proteins of the animal tissues are broken down into simpler ones resulting in softening and greenish discoloration of the meat. These autolytic changes include proteolysis and fat hydrolysis which are pre-requisites for microbial decomposition. Excessive autolysis is termed souring. This makes 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 flavor.

### 2.5.2 Shelf-life of beef and the influencing factors.

Shelf-life of a food product is defined as the period of time it takes before the product's color, flavor, texture, aroma and nutritional value become deteriorated to the point that the product no longer becomes acceptable and loses its nutritional value (Singh and Anderson, 2004). The IFST guidelines (1993) defines it as the period of time during which the food product will remain safe; retain its desired sensory, chemical, physical and microbiological characteristics as well as comply with any label declaration of nutritional data, when stored under recommended conditions. The shelf-life of meat is the amount of time that passes before meat becomes unpalatable or unfit for human consumption because of the growth of spoilage organisms, or

more specifically, the time required for spoilage organisms to reach an unacceptable level (Delmore, 2009). This growth of spoilage organisms renders the product organoleptically undesirable but not necessarily unsafe. Shelf-life properties may include appearance, texture, flavor, colour, and nutritive value (Singh and Singh, 2005).

A number of interrelated factors influence the shelf-life and keeping quality of meat, which are: holding temperature, atmospheric oxygen, pH value, water activity, indigenous enzyme moisture (dehydration), light and most importantly, microorganisms. All of these factors, either alone or in combination, can result in detrimental changes in the color, odour, texture and flavor of the meat. (Zhou *et al*., 2010).

a. **pH and water activity value:** Generally speaking, the lower the pH - value and/or water activity ($a_w$) - value, the longer the shelf-life of meat and meat products. Both factors (either pH or $a_w$ alone or the two together) have a decisive influence on the growth of micro-organisms in food. The varying pH of animal carcasses can be said to be due to the stress caused by pre-slaughter handling of animals which results in the anaerobic depletion of their glycogen level leading to the production of lactic acid (Miller, 2002). The ultimate value pH of beef is said to be 6.2 (Addis, 2015). Long-term stress before slaughter or starvation uses up the glycogen so that less lactic acid is formed after slaughter resulting in an abnormal muscle condition in which it remains dark purplish-red on exposure to air instead of a bright red color. The resulting higher values of pH (6.4 - 6.8) results in ‘dark cutting beef ’ or dark, firm and dry (DFD) beef which has a shorter shelf-life as opposed to pale, soft and exudative meat (PSE) which has a pH lower than 6.2 (5.4 - 5.6) (Miller, 2002). The PSE meat, however, has a poor flavor and it is difficult to use by butchers or meat processors. Allen *et al*, (1997) observed a correlation between

pH and color stating that darker meat fillets have a shorter shelf-life than lighter ones due to their differences in pH. Blixt and Borsch (2002), also confirmed this observation when they carried out a study to compare the shelf-life of vacuum-packed pork and beef. They also observed a strong correlation between pH and meat spoilage. *Enterobacteriaceae*, *Brochothrix thermosphacta and Pseudomonas* have been seen to thrive on meat with high pH value even under vacuum packaging (Rousset and Renerre, 1991). Meat with high pH spoils quickly since the low acidity favors rapid bacterial growth.

b. **Oxygen:** The oxygen content in air is about 20%. Oxygen provides conditions that shortens the shelf-life of meat and other food products by providing conditions that enhances the growth of microorganism; cause damage to food with the help of enzymes (peroxidase and catalase), which causes darkening in leafy vegetables; and it can cause oxidation (lipid oxidation) which causes rancidity and off-flavors in meat. An increase of oxygen causes a significant enhancement of oxidation, decrease of shelf-life due to discoloration and off-odor development (Martinez *et al.*, 2006). In order to curtail the effect of oxygen on the shelf-life of beef, variations of meat packaging systems used during transportation, distribution and storage, have been employed (Realini and Marcos, 2014). Fresh meat can either be packaged in air, using an oxygen-permeable film; in a vacuum, where all the air is removed; or by modifying the atmosphere of the meat usually by altering the gas concentrations before storage (Baxter, 2000). Meat packaged in air ($O_2$) reduces meat shelf-life based on high lipid oxidation and poor quality. However, at the initial stage, meat exposed to oxygen seems to improve meat color via surface lightness and redness; but this decreases with increasing days of storage (Lorenzo

and Gomez, 2012; Martinez *et al.*, 2006; Seydim *et al.*, 2006). Vacuum packaging (packaging in the absence of $O_2$), leads to the extension of shelf-life in terms of both color and odor stability due to low oxidation rates (Brenesselova *et al.*, 2015; Seydim *et al.*, 2006). Shelf-life and quality of fresh red meat can be extended by using modified atmosphere packaging (MAP) coupled with refrigerated storage (Leygonie *et al.*, 2012). MAP with elevated levels of carbon dioxide and reduced levels of oxygen and the use of oxygen scavengers inhibit the growth of various aerobic spoilage bacteria and prolong color stability of fresh red meat (Arteaga and Millis, 2014; Saucier, 2000). Various concentrations of $CO_2$ have been found to increase shelf-life of meat. For instance, low $CO_2$ concentrations are best for improving the keeping quality of pork and extended its shelf-life (Martinez *et al.*, 2005; Zhang and Sunder, 2005). Rousset and Renerre (1991) found out that high $CO_2$ concentrations improved shelf-life of meat with high pH for up to 42 days. Other combinations of CO and $N_2$ have also been seen to influence the shelf-life of meat by reducing aerobic plate counts, lactic acid bacteria and *Enterobacteriaceae* counts (Insausti *et al.*, 2001; Sorheim *et al.*, 1997).

c. **Light:** The color pigment of meat is called myoglobin and when exposed to oxygen, it oxidises to oxymyoglobin ($MbO_2$) which gives the meat its bright red color. This color makes meat attractive to purchasing consumers (Suman *et al.*, 2014). However $MbO_2$ is easily discolored to brown metmyoglobin (MetMb). Light plays a critical role in pigment photooxidation, since it catalyzes MetMb formation (Renerre and Labadie, 1993). Ultraviolet (UV) light has been seen to catalyse the effect of MetMb formation. According to Djenane *et al.* (2001), lighting without UV radiation led to a significant

delay of meat spoilage while the shelf-life of fresh meat was extended by using either a low UV lamp or a fluorescent with a UV filter. Also, the prolonged exposure of meat and meat products to daylight or artificial light accelerates oxidation and rancidity because light provides the energy for these processes (Chambers and Grandin, 2001).

d. **Temperature:** When temperature is not properly controlled, meat will spoil. An increase in temperature doubles the rate of chemical reactions due to an increased rate of natural enzymes which will cause protein to breakdown or denature; emulsions to break; some moisture will be lost, some vitamins will be destroyed, and color, flavor and odor will be affected (Fraser, 2012, Warner *et al*., 2014)). Microorganisms rapidly proliferate at elevated temperatures and slime development is a definite visual sign of microbial growth (FAO, 1991). Also, exposing meat to uncontrolled cold temperatures will also cause physical spoilage. Bacteria relevant to meat, meat products and other food are divided into three groups according to the temperature range within which they can grow: mesophiles 10 – 45 °C; psychrotrophs 10 – 45 °C, or slow growth at 0 –10 °C. Mesophiles will not grow below 10 °C but psychrotrophs, of which Pseudomonas are the more important, will grow down to 0 °C. The nearer to 0 °C of the storage temperature, the slower the growth of the spoilage bacteria and the longer the shelf-life of meat (FAO, 1991).

e. **Microorganisms:** Microorganisms, specifically bacteria, molds, and yeasts, can cause meat to spoil. Bacteria break down protein in meat (proteolytic bacteria) and cause strong off-odors, slime formation and discolouration in meat. Microbial growth on meat will cause meat spoilage at a total viable count (TVC) of $10^6/cm^2$ because of off-odours.

Slime and discoloration appear at $10^8/cm^2$ (FAO, 1991). Various types of bacteria are found on meat stored in air under refrigeration. Pseudomonas is an aerobic psychrotrophic bacteria which causes putrefactive odors and slime formation in meat. *Enterobacteriaceae* cause putrefactive spoilage both in the presence and absence of oxygen (Sentence, 1991). Lactic acid bacteria such as *Lactobacillus* are gram-positive bacteria which also cause spoilage and grow readily in the absence of oxygen. Other organisms responsible for meat spoilage consist of *Brochotrix thermosphacta, Carnobacterium, Clostridium, Enterobacterium, Leuconostoc,* and *Alteromonas*

## 2.6 Food Preservation Methods

Fresh meat is a highly perishable product due to its biological composition. Many interrelated factors influence the shelf life and freshness of meat such as holding temperature, atmospheric oxygen ($O_2$), endogenous enzymes, moisture, light and most importantly, micro-organisms (Zhou *et al*., 2010). Microbial growth, oxidation and enzymatic autolysis are three basic mechanisms responsible for spoilage of meat (Dave and Ghaly, 2011). With the increased demand for high quality, convenience, safety, fresh appearance and an extended shelf life in fresh meat products, proper preservation techniques have been employed.

1. **Canning** is the process of heating the product at a specified temperature for a specific length of time (pasteurizing), and then vacuum sealing the pasteurized food in special glass jars designed for this purpose (Erdogdu and Tutar, 2011). It can be used with most foods, including fruits, vegetables, meats, seafood, and some prepared foods (Kapica and Weiss, 2012). Canning requires the purchase of reusable canning jars and rings, one-time use sealing lids, and some practice to learn the necessary and detailed steps. Canned beef

sausages has been reported to be of good quality and safe if processed without added nitrite and retorting at 107.2 ºC for 80 minutes. It also has better palatability characteristics at least for 3 months of storage at room temperature (Maha and Abogroun, 2013).

2. **Freezing** Freezing and thawing are complex processes that involve heat transfer as well as a series of physical and chemical changes which can affect the quality of the product (Bing and Da-Wen, 2002). A very important aspect in meat industry – especially from a financial point of view – is the drip loss after thawing. Studies have demonstrated the link between drip loss, freezing speed and aging rate of the freezing meat (Gambuteanu *et al*., 2013). Therefore, Anon and Calvelo (1980) showed the influence of the freezing rate on the formation of ice crystals that underlies the mechanisms of damage during the freezing of meat tissue. The formation of ice crystals during freezing damages the ultrastructure and concentrates the solutes in the meat which, in turn, leads to alterations in the biochemical reactions that occur at the cellular level and influence the physical quality parameters of the meat (Leygonie *et al*., 2013; Jo *et al*., 2014).

3. **Chilling:** The storage of food using low temperatures is by far the most commonly used measure of preservation in fresh foods production and distribution. Meat is recognized as one of the most perishable foods, and refrigeration temperatures (4 ºC) are always used to delay the spoilage of fresh meat. The low temperatures are used for the storage of carcasses after slaughter, subprimal cuts and during transport of meat to the distributors, and finally for the storage of meat at the various retail sites. During all of these phases the chill temperature is used to delay meat alteration through the development of spoilage bacteria (Ercolini *et al*., 2009).

4. **Drying** is the process of dehydrating foods until there is not enough moisture to support microbial activity. It can be used with most foods, including fruits, vegetables, meats, seafood, grains, legumes, and nuts. There are several different techniques, some are relatively easy to do and require no special equipment (Hui *et al*., 2004). Reducing the sample thickness or increasing the transferred heat, by increasing the temperature or velocity of the drying medium, result in accelerating the drying process. Temperatures above 160 °C, however, cause changes within the meat and its surface and prevent the bound moisture from getting to the meat's surface to evaporate (Speckhahn *et al*., 2010). Acidified predrying treatment has high pathogen inactivation during drying, regardless of drying temperature (Yoon *et al*., 2006). The use of modified marinades may improve the effectiveness of drying in inactivating *L. monocytogenes (*Calicioglu *et al*., 2002). Modified marinades and low water activity provide antimicrobial effects against possible post-processing contamination of beef jerky with *E. coli* O157:H7. Acid adaptation of cultures enhances their inactivation during storage (Calicioglu *et al*., 2003).

5. **Dry salting** Salt is one of the most widely used additives in food industries because of its low cost and varied properties. It has a preservative and antimicrobial effect as a direct consequence of the capacity of sodium chloride to reduce water activity values. In addition, sodium chloride is a flavour enhancer as a consequence of its effect on different biochemical mechanisms. It also has flavour enhancing effects from reducing or enhancing the enzymatic activity of some enzymes responsible for the development of different organoleptic parameters (Albarracín *et al*., 2011). Drying, when combined with dry salting, leads to higher salt content at the equilibrium and allows more dehydration in the product. There is absence of true lactic fermentation after salting and drying,

confirmed by small change in pH and lactic acid bacteria proliferation during salting and drying (Chabbouh *et al*., 2011). Dry salting during 24 h is the most suitable salting method for meat elaboration since it allows the highest dehydration level, preserves microbial quality and induces proteolysis which could generate aromatic compounds (Chabbouh *et al*., 2012).

6. **Curing** is similar to pickling, and uses salt, acid, and/or nitrites. It is used for meat and fish. Simple, modern curing methods often reduce the amount of salt and nitrites, which may require that you refrigerate or freeze the final product. Shelf-stable products require the use of adequate amounts of nitrites and a complex drying process using special equipment and exacting technique. Some curing methods also employ a secondary process such as fermenting, smoking, or sealing.

   During curing, moisture content and water activity decreased as the concentration of salt in the curing solution increased, presumably due to osmotic dehydration. Moisture content, water activity and pH reduced further during storage (Okonkwo and Anyaene, 2009).

7. **Smoking** is a complementary process to curing that improves flavor and appearance, and can also act as a drying agent. Smoked meats are less likely to turn rancid or grow mold than unsmoked meats.

8. **Sealing** is a process of covering food to keep out air, which *delays* (but does not stop) the activity of spoilage organisms. It is used primarily as a complementary process to other methods such as drying or freezing. Both fat sealing and vacuum sealing methods are relatively easy. Vacuum sealing uses a relatively inexpensive small appliance.

9. **Cellaring** is the process of storing foods in a temperature-, humidity-, and light-controlled environment. It can be used with many foods, especially vegetables, grains, and nuts, as well as fermented foods and dry-cured meats. There are many different methods for cellaring food, all of which are relatively easy to do. Some require simple, inexpensive equipment and it is a feasible technique which can be practiced to some degree.

10. **Chemical Methods** employ preservatives which are substances used to extend the shelf-life of meat by reducing microbial, enzymatic and oxidative spoilage/ proliferation during slaughtering, transportation, processing and storage (Addis, 2015). They are however not to be used as a substitute for poor processing or to cover up an already spoiled meat (Ray, 2004). They offer a good protection for meat in combination with refrigeration. Common antimicrobial compounds include chlorides, nitrites, phosphates, sulfides and organic acids (Chipley, 2005).

## 2.7 Decontamination Interventions

There is an increasing interest in effective decontamination treatments because healthy food-producing animals can harbor food-borne pathogens and the complete prevention of contamination during slaughter can hardly be avoided (Loretz *et al*., 2011). There are various intervention techniques targeted at reducing or eliminating bacteria that may be human pathogens as well as those that may cause meat spoilage. They include steam / hotwater washing, chemical rinsing, ionizing radiation.

a. Steam has been shown to be effective in reducing the number of microorganisms on meat surfaces ((Barco *et al*., 2015; Hassan *et al*., 2015; Loretz *et al*., 2011). Immersion in hot

water is also one of the many potential methods for reducing levels of pathogenic bacteria on the surface of meat (Goksoy *et al*., 2001). Hot water reduced *E. coli* O157 prevalence by 81 %. However, hot water only works if proper temperature is maintained to get 74˚C on the carcass surface for >5 s (Koohmaraie *et al*., 2005).

b. Electron beam irradiation could be useful in improving the microbial safety without impairing the quality of beef jerky during storage (Kim *et al*., 2010). Since under normal processing conditions the levels of these pathogens on beef carcasses would be lower than the lethality caused by the treatment used, irradiation at 1 kGy would be expected to eliminate the hazard represented by verotoxigenic *Escherichia coli* (Kundu *et al*., 2014). However, irradiating fresh meat, even at low doses, can result in off-odors and flavors. This detrimental effect can be reduced by oxygen exclusion (vacuum packaging), replacement with inert gases (nitrogen), addition of protective agents (antioxidants), and post-irradiation storage to allow flavor to return to near-normal levels (re-packaging or double packaging in oxygen permeable film) (Brewer *et al*., 2009).

c. Chemical rinsing is a technique employed by most commercial plants where decontaminants are applied to meat surfaces via spray rinsing cabinets through which carcasses are passed automatically. They can also be applied via dipping treatments. The most frequently used chemical decontaminants are solutions of organic acids (1 - 3%), such as acetic and lactic acids, which reduce numbers of bacteria on carcass tissue (Sofos *et al*., 1999). Such organic acids are most useful as warm (50 - 55 $^{o}$C) rinses, applied before chilling, especially in combination with preceding treatment using hot water or

steam (Gorman *et al*., 1997). In addition to organic acids, several other chemical solutions have been proposed and tested (some have been approved) for use in decontamination systems. Such chemicals include common chlorine and chlorine dioxide, trisodium phosphate, hydrogen peroxide, sodium hydroxide, ozone, sodium bisulfate, sodium chloride, acidified sodium chlorite, nisin, potassium sorbate, cetylpyridinium chloride, and activated lactoferrin (Dickson *et al*., 1994).

### 2.7.1 Lactic acid

Lactic acid (2-hydroxypropanoic acid) also known as milk acid is a liquid, colorless, water-soluble, organic acid with a chemical formular $C_3H_6O_3$ that is produced both naturally and synthetically. Lactic acid is the bitter-tasting fermentation product of bacteria interacting with lactose (milk sugar) and it is the most common acid constituent of fermented milk products, occurring naturally in sour milk, yogurt, cheese, cream, and buttermilk. According to Leroy and Vuyst (2004), the production of fermented foods is based on the use of starter cultures, for instance lactic acid bacteria (LAB) that initiate rapid acidification of the raw material. Lactic acid also occurs in the form of its salts (lactates) in muscles and blood during exercise, as a result of the metabolism of glycogen for energy. Its application is widely used in food, pharmaceutical, textile, leather and chemical industries. In food, it is used as a food preservative, curing agent and flavouring agent. It is an ingredient in processed foods and it is used as a decontaminant in meat processing with a generally regarded as safe status (GRAS) (USFDA, 2013).

As a decontaminant, lactic acid results in the significant reduction of a bacterial flora not only by means of a pH drop but also by a specific action of the acid in the undissociated form (Smulders

*et al*., 1986). The lipophilic nature of the undissociated acid allows it to diffuse across the cell membrane collapsing the electrochemical proton gradient. In addition to this antimicrobial property, it can also act as a potentiator of the effects of other antimicrobial substances by functioning as a permeabilizer of the Gram-negative bacterial outer membrane (Alakomi *et al*., 2000). The salt of lactic acid (lactate) appears to be bacteriostatic and their ability to control spoilage and pathogenic bacteria in fresh and processed meat favors their use, particularly in refrigerated meat products and in combination with other microbial inhibitors (Shelef, 1994). Using the right dosage, lactic acid provides an appropriate pH which enforces its bactericidal properties while not adversely affecting the sensory attributes of food. Concentrations of 1 % v/v with a pH of 2.4 does not cause discoloration of meat surfaces and up to 2 % does not cause off-flavors in meat. Both concentrations result in significant reduction of bacterial flora (Baysal and Unluturk, 2005; Beyaz and Tayar, 2010 ). According to Smulders and Greer (1998), dilute solutions of organic acids (1 to 3 %) are generally without effect on the desirable sensory properties of meat when used as a carcass decontaminant. However, dependent on treatment conditions, lactic acid can produce adverse sensory changes when applied directly to meat cuts, with irreversible changes in appearance being a frequent occurrence. A considerable literature reports and confirms the antibacterial efficacy of dilute solutions of lactic acid (Belanger and Stelzleni, 2014; Brustolin *et al.*, 2014; Castillo *et al*., 1999; Naveena *et al*., 2006; Okolocha and Ellerbroek, 2005; Pfeifer *et al.*, 2014; Pipek *et al*., 2006; Van Netten *et al.*, 1995; Zeitoun *et al*., 1992).

### 2.7.2 Trisodium phosphate

Trisodium phosphate (TSP) is an inorganic compound with the chemical formular $Na_3Po_4$. It is a white, granular or crystalline solid, highly soluble in water producing an alkaline solution. They

are used as food additives, cleaning agents, lubricant, stain remover and degreaser (Schrodter *et al.*, 2008). They are also industrially used as raw materials in manufacturing products such as toothpastes (including baby toothpaste), mouthwash, hair coloring and bleaching products. As food additives, they are used in processed meat, processed cheese, canned foods and as leavening agent in commercial cakes and baked goods. As food preservatives, they act as antimicrobials and are generally recognized as safe (GRAS) by the Food and Drug Administration and has been approved by the U.S. Department of Agriculture for use as a food ingredient (Branen *et al*., 1990). According to Skovgaard (1996), TSP is naturally present in tissues and bones and may thus be considered to be harmless. Possible modes of action of TSP include:

(i) Exposing microorganisms to high pH, which might particularly affect cell membrane components (Mendonca *et al*., 1994).

(ii) Enhancing detachment of bacteria from food surfaces by sequestration of metal ions (Lee *et al*., 1994).

(iii) Removing fat from the skin surface, thereby allowing bacteria to be washed from the food surfaces more effectively (Bender and Brotsky, 1992; Giesse, 1992; Kim and Slavik, 1994). According to Sampathkumar *et al*. (2003), TSP treatment permeabilizes and disrupts the cytoplasmic and outer membranes of serovar Enteritidis cells because of its alkaline pH, which in turn leads to release of intracellular contents and eventual cell death. Application of TSP at high concentrations are necessary because low concentrations of TSP have been found to favor the growth of unwanted microorganisms (Capita *et al*., 2001). At 8 to 15% (wt/vol), TSP has been demonstrated to reduce the number of gram-negative pathogens surviving on surfaces of foodstuffs (Ahmed and El-Atti, 2010; Dorsa *et al.*, 1997; Capita *et al*., 2000; Loretz *et al*., 2010; Okolocha and Ellerbroek, 2005). This can be applied via spraying or dipping. The sensory

analysis of TSP used on carcasses, in terms of acceptability, color, smell, flavor, texture and taste can be found within an acceptable range of 8 to 10 % (Capita *et al.*, 2000; Okolocha and Ellerbroek, 2005; Pohlman *et al.*, 2002). Thus, TSP is said to be an acceptable decontaminant when used in the right concentrations.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Culture media

Media were obtained from Oxoid (Basingstoke, England) and they comprised of Nutrient agar, MacConkey agar, Eosin Methylene Blue, Buffered Peptone Water, Urease agar, Triple Sugar Iron (TSI) agar, Simmon's Citrate Agar, Sulphide Indole Motility (SIM) agar, Methyl Red Voges Proskauer broth (MRVP)

### 3.1.2 Laboratory materials

Wooden stands with suspended hooks, Pressure sprayers (Tecto, China), Conical flasks, Test tubes, Pipettes, Disposable petri dishes (PlastiLab, Lebanon), Disposable hand gloves, Sample bottles, Aluminum foil, Bijou bottles, Distilled water, Masking tapes, Permanent markers, Polythene bags, Coolman boxes, Pipettes aspirator, Cotton wool, Measuring cylinders

### 3.1.3 Chemicals/reagents

Lactic acid (Analar$^{R}$, England), Trisodium phosphate (Sigma-Aldrich, Germany), Urea, Kovac's reagent, Tryptophan deaminase, Methyl red, Voges proskuer 1, Voges proskuer II, Methanol, Sodium chloride

### 3.1.4 Rapid kits

Microbact$^{TM}$ 12A Identification Kits for *Enterobacteriaceae* (Oxoid, Basingstoke, England).

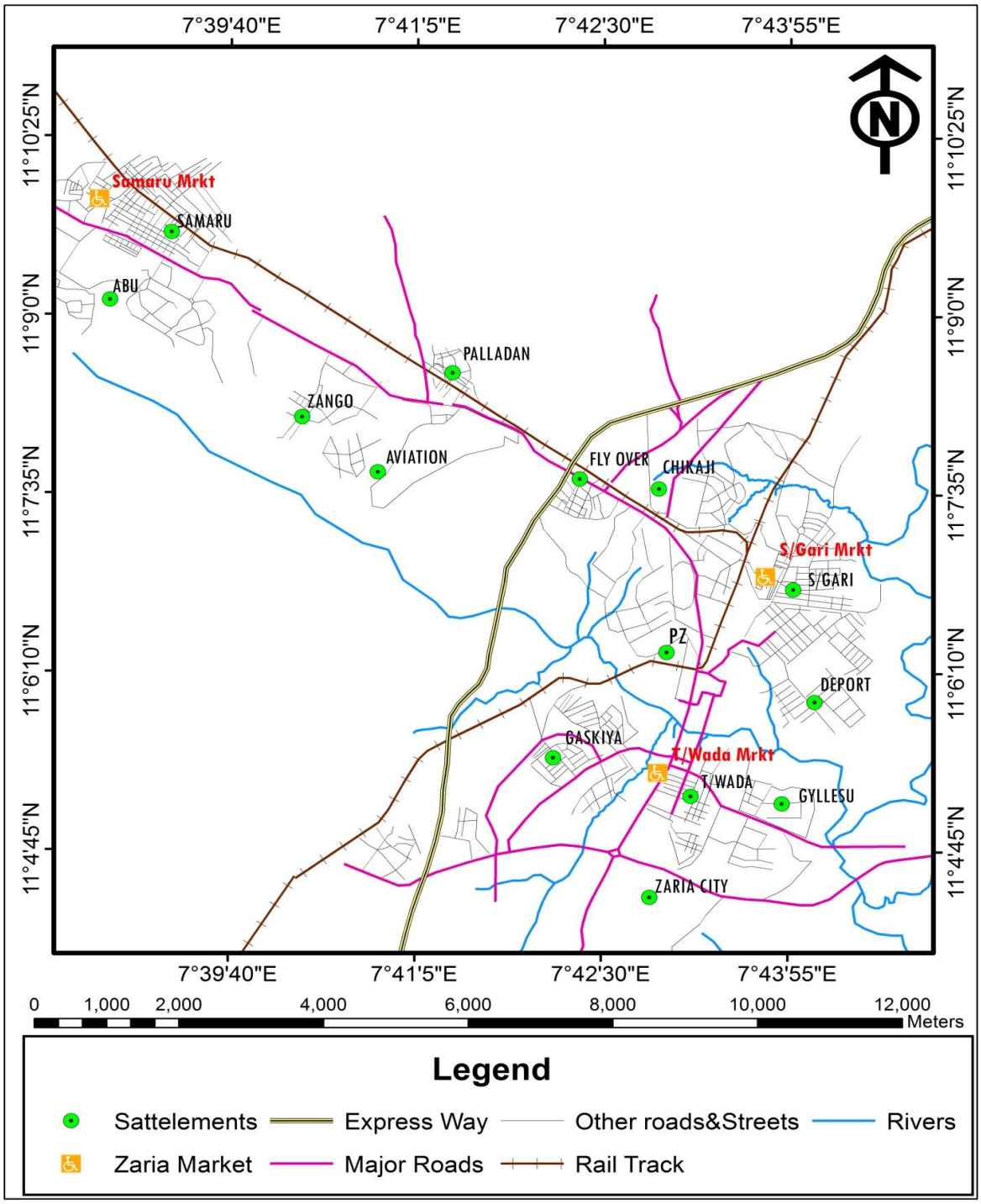
### 3.1.5 Laboratory equipment

Incubator (Astell Scientific, UK), Refrigerator (Thermocool, Nigeria), Hotbox oven (Gallenkamp, England), Electronic Ohaus balance, Bunsen burner, Test tube racks, Autoclave (Gallenkamp, England), Pasteur loop, Pasteur pin, Syringes, Water bath (Kottermann Labortechnik, Germany), Glass rod, Glass hockey stick.

## 3.2 Study Area

The study was carried out in Zaria metropolis of Kaduna State located within latitudes 11$^{o}$10$^{/}$N and longitude 07$^{o}$38$^{/}$E. It is a medium-sized city made up of two Local Government Areas, namely Zaria and Sabon Gari. It has an estimated population of 547,000 and a growth rate of 3.5% per annum (KSG, 2008). The city is located within semi-arid and sub humid regions. Kaduna State is situated in the North West geopolitical zone of Nigeria. Climate type comprises a rainy season (about 6 months). The rainy season is preceded by mean maximum daily temperature of between 35 $^{o}$C and 40 $^{0}$C. Annual total rainfall is about 1,272mm and rain falls mostly from May to October. Agriculture is the main-stay of the economy of Zaria with about 80% of the people actively engaged in agro farming. Another major occupation of the people is animal rearing, horticulture and poultry farming. The animals reared include cattle, sheep, goats and pigs (Mamman, 2000). Operational abattoir within the study area is the Zango abattoir.

Slaughter slabs are located at Hayin Dogo, Paladan and Tudun-wada. The major meat markets include Samaru, Tudun-wada and Sabon-gari markets. Meat can also be purchased at random locations in meat stalls or in kiosks within Zaria. Zaria also harbors a lot of higher and educational institutions (Academic or professional).




Source**:** Zaria Quirk Bird Image (2015)

**Figure 3.1: Map of Zaria metropolis showing the three different markets (Samaru, Tudun- wada and Sabo markets).**

## 3.3 Procurement of Beef Samples

A total of two hundred and sixteen (216) beef samples, 100 g each were used. These were obtained from 3 retail markets located in Samaru, Tudun-wada and Sabon-gari respectively. Seventy-two (72) beef samples were obtained per market. Samples were collected from any part of the carcass in a form readily available to purchasing consumers. These samples were obtained using the convenience sampling procedure. Samples were collected weekly and transported on ice to the Bacteriology laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, A.B.U, Zaria for further processing.

## 3.4 Treatment of the Beef Samples

### 3.4.1 Chemicals

The following acid and alkaline substances were used: 1 % lactic acid (Analar$^{R}$, England) and 10 % Trisodium phosphate (TSP). These substances were used in a 1-litre (1L) and 2-litre (2L) spray treatments.

### 3.4.2 Spraying

A 1- liter and 2- liter hand pressure or trigger sprayer with an even spray jet of pressure were used for spraying. The beef samples were treated as follows:

A total of seventy-two (72) pieces of beef samples, 100 g each, were sprayed with lactic acid; 24 samples from each market. Twelve (12) pieces were sprayed with the 1L spray treatment and another 12 pieces with the 2L spray treatment. Trisodium phosphate (TSP) was used in treating another 24 pieces, 12 pieces with 1L spray and another 12 pieces with the 2L spray solution. Twenty four pieces served as control (spray treatment with distilled water only), 12 for the 1L spray and another 12 pieces with the 2L spray treatment. The duration of spray was 10s, after which a dripping time of 5mins was allowed. The beef samples treated with TSP were re-sprayed

with water for the same duration as the spray treatment (10s). This was done in order to enhance acceptability of the beef sprayed with them due to the high alkalinity of the TSP solution at that concentration (10 %). All procedures were aseptically carried out. In spraying, the beef samples were suspended on hooks hung on a wooden stand. Spraying was done on all sides.

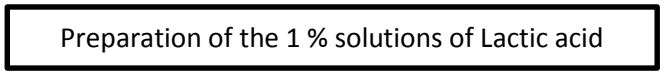
### 3.4.3 Storage

As each batch of treatment was completed, beef samples were wrapped with sterile polythene bags, labeled appropriately, stored and maintained at 4 $^{O}$C in the laboratory until analysed. The sampling days were normally days 0 (the day of collection), 3 and 6.

### 3.4.4 Sampling

Each beef sample was washed using the whole carcass rinse method by pouring sterile buffered peptone water whose volume was usually half the weight of the meat (50 ml) into sterile polythene bags, securing the mouth and shaking gently vertically and horizontally for about one minute. 10 ml of the wash solution was used as test solution for the microbiological analyses. This procedure was applied to each treatment (Okolocha and Ellerbroek, 2005).

A flow chart diagram summarizing procedure is further illustrated in Figures 3.2 and 3.3



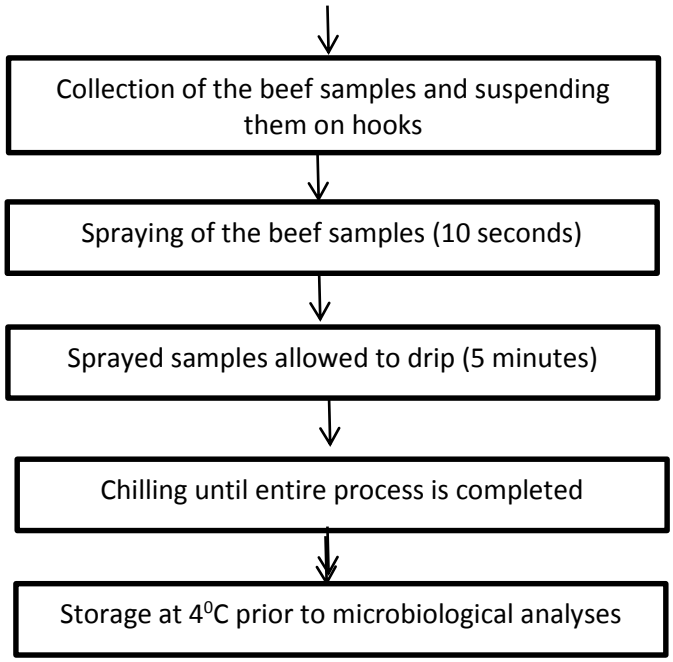



**Figure 3.2: Flow Diagrams summarizing the Spraying Operations using Lactic acid**

Preparation of a 10 % TSP solution

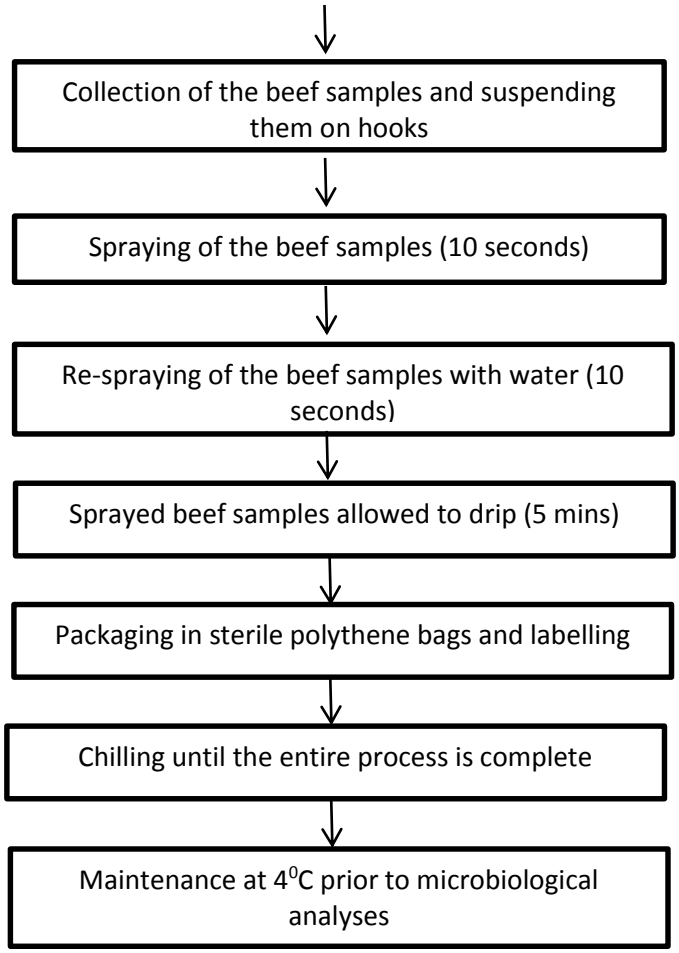



**Figure 3.3: Flow Diagrams summarizing the Spraying Operations using Trisodium Phosphate**

## 3.5 Microbial Evaluation

### 3.5.1 Total aerobic plate count

Total aerobic counts were carried out on days 0, 3 and 6 of storage on all treated (i.e. with LA and TSP) and control groups of the beef samples. Nutrient agar was used to achieve total aerobic count. A 100-fold serial dilution was carried out using a sterile micropipette to aspirate 0.1 ml of the wash solution and then dispensed into 9.9 ml physiological saline. Serial dilutions were up to $10^7$ dilution factor. The nutrient agar plates were inoculated with 0.1 ml of the last tube. This inoculum was uniformly spread using sterile glass hockey stick. The inoculated plates were then incubated at 37 $^{O}$C for 24hrs, after which the colonies were counted.

### 3.5.2 Coliform counts

Total coliform counts were also carried out on all treated and control groups of the beef samples on all days of storage (i.e. days 0, 3 and 6). MacConkey agar was used to obtain coliform plate counts. Hundred-fold serial dilutions were carried out and were used to inoculate the prepared MacConkey plate. A sterile glass hockey stick was used to spread the inoculum. The inoculum was incubated for 24hrs at 37˚C and afterwards was counted.

### 3.5.3 Isolation of *E. coli*

Ten (10) ml of the wash solutions pre-enriched in buffered peptone water was aspirated onto a sterile tube and was incubated at 37 $^{o}$C for 24hrs for the isolation of *E. coli*. A loop-full was streaked on Eosin methylene blue (EMB) agar plates. The inoculated plates were incubated at 37 ˚C for 24hrs. The plates were observed after 24hrs for characteristic greenish metallic sheen. A loop-full of the suspected isolate was then inoculated on nutrient agar slant and stored for further biochemical analyses (Bacteriological Analytical Manual, 2002).

### 3.5.4 Biochemical confirmatory tests

The suspected *E. coli* isolates were further subjected to biochemical tests by testing their ability to ferment sugar (lactose and sucrose) using Triple sugar ion (TSI) agar, Simmon's citrate agar, Sulphide indole motility agar (SIM), Urease agar and Methyl red voges proskauer broth. The presumptive isolates were run on Microbact 12A kit for *Enterobacteriaceae* (Oxoid, Basingtoke, England) for further biochemical confirmation.

## 3.6 Sensory Analyses

The sensory evaluation of both the treated beef samples and controls was based on appearance, smell and taste. The triangle test method was used. This was conducted based on the work of Meilgaard *et al*. (1999). Seven (7) panelists were asked to determine the odd sample between two similar samples and one different sample. Two samples were labeled as treated beef samples while one was labeled as control. The labels were made in a way to conceal the true identity of the samples from the panelists. These procedures were carried out during evaluations for each of the treatments. Cooked (roasted) beef samples were evaluated for appearance, smell and taste while raw beef samples were evaluated for only appearance and smell. The evaluations were based on levels of preference.

For the roasted beef samples, thin slices (100g) each, were washed, allowed to drip water and then placed on a grill using low heat (coal). Little quantities of groundnut oil and salt were sprinkled on each side of the meat after which the beef was allowed to cook (roast) for about 30 mins, turning each side at intervals so that each side of the meat would be allowed to roast properly.

### 3.6.1 Questionnaire

A sensory analyses form was administered to each panelist. Panelists were asked to evaluate for fresh beef samples, roasted beef samples and the evaluation of cut pieces. Results were presented in percentages using Microsoft Excel for the number of panelists showing preference.

### 3.7 Statistical Analyses

The data collected from the microbial evaluation were subjected to a series of statistical procedures aimed at demonstrating differences between bacterial counts on the beef samples on one hand and the result of the treatments with the different chemicals using different modes (1-1 spray treatment, 2-1 spray treatment) at different storage days obtained from different markets. Therefore, the multi-factor Analysis of Variance (ANOVA) was performed based on the following factors: markets where samples were obtained, treatments (control, lactic acid, and trisodium phosphate), modes of treatment (1-1 spray treatment, 2-1 spray treatment) and days of storage (0, 3 and 6). This was followed by the Tukey's multiple range tests. Data were analysed using Statistical package for Social sciences (SPSS) version 20, Graph pad Prism version 5.0 and Microsoft Excel. Results obtained for the frequency of *E. coli* isolation were also analysed using SPSS version 20. Values of $P < 0.05$ were considered significant.

## CHAPTER FOUR

## 4.0 RESULTS

The raw data, questionnaire format for the sensory evaluations and all the statistical analyses done in this work are given on the appendix (pages 106-118).

### 4.1 Mean Log Reductions

Table 4.1 shows the mean log reductions in aerobic plate counts (APC) and the total coliform counts (TCC) following the different treatments with 1% Lactic acid (LA) and 10% Trisodium phosphate (TSP) at the three different markets (Samaru, Tudun-wada and Sabo).

### 4.1.1 Mean log reductions of aerobic plate count for Samaru market

The effect of the 1 liter spray treatment with LA was seen only to be effective at the third day of storage while decontamination effects were minimal on beef samples treated with the 2 liter spray treatment only at the sixth day of storage. Treatment with TSP (1L) showed no reduction effect on the aerobic microbial load on beef samples while the 2 liter treatment gave minimal reductions on microbial load on day 0 only. These reduction effect are further illustrated in figure 4.1.

The multifactor analysis of variance on the $\log_{10}$ transformed values revealed significant differences ($P< 0.05$) between treatments, mode of treatment and days of storage. There was however no significant difference ($p>0.05$) in the interactions between them. This implies that a combination of treatment and mode of treatments did not effect significant log reductions on the aerobic plate count on beef samples in Samaru market.

When comparing treatments by mode (volume), LA (1L) treatments gave better reductions on aerobic microbial load than TSP (1L) treatments on all days of storage with significant differences ($p<0.05$) on days 0 and 3. In comparing the effects between the two volumes of TSP treatments (1L and 2L), the 1L treatment gave better reductions with significant differences at day 0 only. There were however no significant differences ($p>0.05$) observed between treatment and control (See appendix 7).

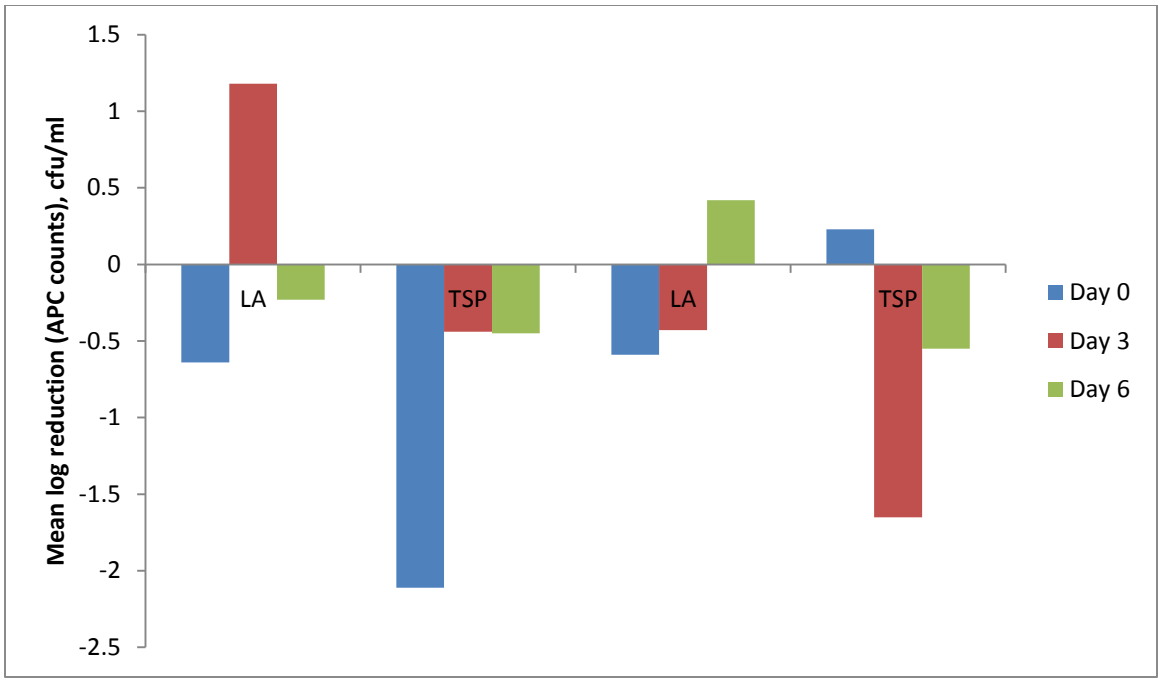



**Figure 4.1: The effects of the different acid and alkaline substances and the different modes of treatment on the bacteriological conditions CFU/ml (Aerobic Plate Count, APC) on beef samples in Samaru Market**

### 4.1.2 Mean log reductions of total coliform count for Samaru market

The 1L spray treatment with LA brought about reductions on all days of storage, however, this reduction effect decreased after the third day of storage. With the 2L spray treatment, the effect increased with increasing days of storage though the increase on the sixth day was quite minimal.

Spray treatment with TSP (1L), showed no reduction effect on the coliform microbial load while the 2 liter treatment gave reductions on day 0 only. See further illustrations in figure 4.2.

The multifactor analysis of variance on the $\log_{10}$ values revealed significant differences ($P< 0.01$) between treatments, modes of treatment and days of storage. There were also significant differences ($p<0.01$) in the interactions between them. This implies that the combination of these factors contributed to the minimal reductions (or otherwise) effect on the meat.

As seen with the Aerobic Plate Counts, LA (1L) gave better reductions than the TSP (1L) treatments on all days of storage with significant differences on days 3 and 6. The 2L treatment with LA gave better reductions only at the 3$^{rd}$ and 6$^{th}$ day with significant differences at the 6$^{th}$ day of storage when compared with the TSP treatment of the same volume (2L). Trisodium Phosphate (2L) treatment gave better reductions than TSP (1L) with statistical significance ($p<0.05$) on day 0 only. There were no significant differences observed between treatments (LA 1L and LA 2L). For both volumes of treatment (1L and 2L) however, LA gave better reductions on the microbial load on meat compared to the control on all days of storage. This however was not statistically significant ($p>0.05$) (See appendix 4).

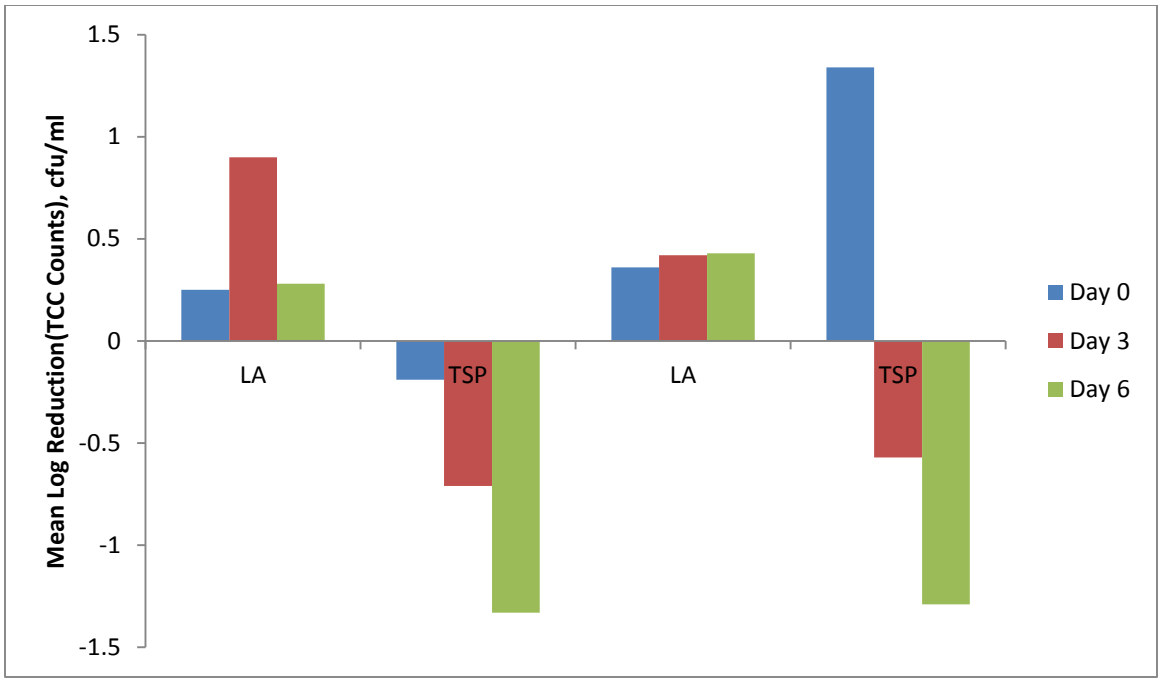



**Figure 4.2: The effects of the different acid and alkaline substances and the different modes of treatment on the bacteriological conditions CFU/ml (Total Coliform Count, TCC) on beef samples in Samaru Market**

4.1.3 Mean log reductions of aerobic plate count for Tudun-wada market

Lactic acid (1 liter spray treatment) had a high reduction effect on day 0 but this decreased with increasing days of storage. The 2 liter spray treatment showed a high reduction effect on day 0 of storage with a decrease and then an obvious increase in reductions from day 3 until the $6^{th}$ day of storage. The TSP treatment had a similar pattern of effect at both modes (1L and 2L) of treatment. There was a consistent decline on the reduction effect of microbial load with increasing days of storage. Further illustrations are shown in figure 4.3.

The multifactor analysis of variance on the $\log_{10}$ transformed values revealed no significant differences ($P>0.05$) between treatments, modes of treatment and their interactions. This implies that the treatment and modes of treatment used were not significant factors in reducing the microbial population on meat. There were however significant differences between the different days of storage. By days of storage, the differences were significant meaning that the mean log reductions obtained varied with the days of storage.

Both modes of treatment (1L and 2L) of LA generally gave better reductions on microbial load than TSP. There was however no statistical significant differences observed between them {LA (1L) and TSP (1L); LA (2L) and TSP (2L)}. Lactic acid (both 1L and 2L) generally gave more effective reductions on microbial load on beef samples compared to the control on all days of storage. There was however no significant difference recorded between them except the LA (1L) treatment which recorded a significant difference on day 0 of storage when compared with the control at the same volume (See appendix 8).

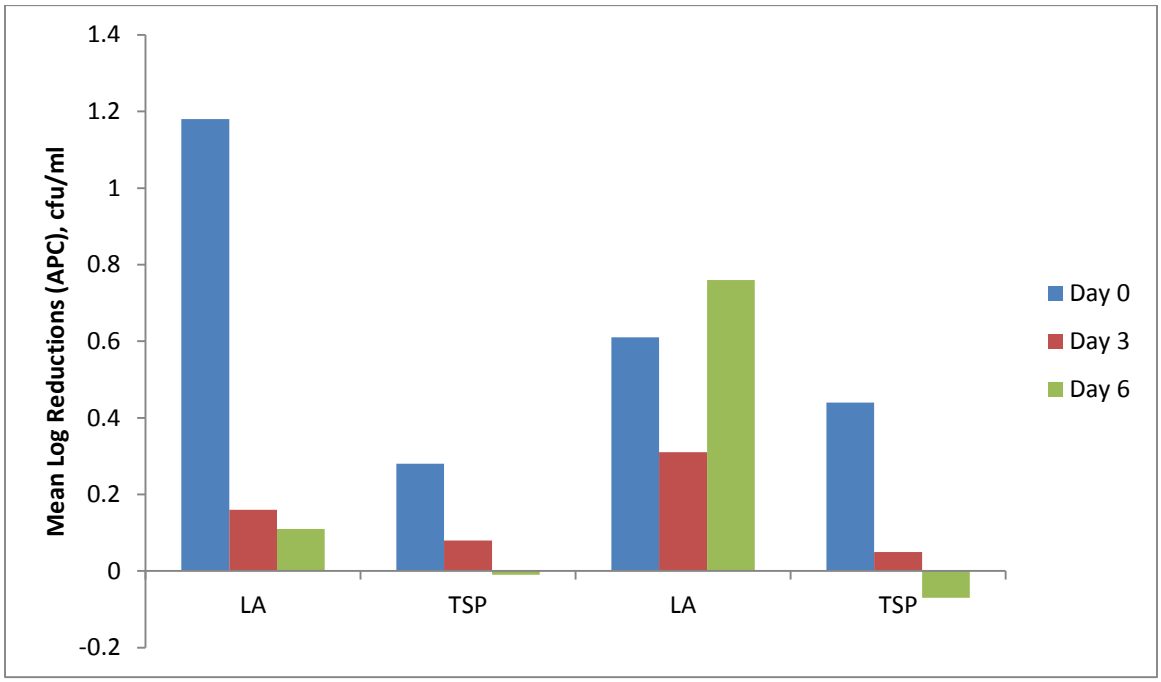



**Figure 4.3: The effects of the different acid and alkaline substances and the different modes of treatment on the bacteriological conditions CFU/ml (Aerobic Plate Count, APC) on beef samples in Tudun-wada Market**

4.1.4 Mean log reductions of total coliform count for Tudun-wada market

The 1 liter spray treatment of LA was effective only at day 0 of storage. Though, the 2 liter spray treatment gave better reductions than the 1 liter spray treatment of LA at days 0 and 3 of storage, this however decreased with increasing days of storage. The TSP (1L) spray treatment gave minimal reductions on day 0 of storage while the 2 liter spray treatment gave reductions only at the third day of storage. Further illustrations are shown in figure 4.4.

The multifactor analysis of variance on the $\log_{10}$ transformed values revealed no significant differences ($P>0.05$) between treatments, modes of treatment and their interactions. This implies that the treatment and modes of treatment used were not significant factors in reducing the microbial population on beef samples. There were however significant differences between the different days of storage. By days of storage, the differences were significant meaning that the mean log reductions obtained varied with the days of storage.

There was no significant difference ($p>0.05$) between treatments {LA (1L) and LA (2L); LA (1L) and TSP (1L); TSP (1L) and TSP (2L)}. Lactic acid (2L) gave better reductions on microbial load on beef samples than the TSP (2L) treatment with significant differences ($p<0.05$) on day 0 only. There was also no significant differences ($p>0.05$) observed between the treatment and the control group (See appendix 5).

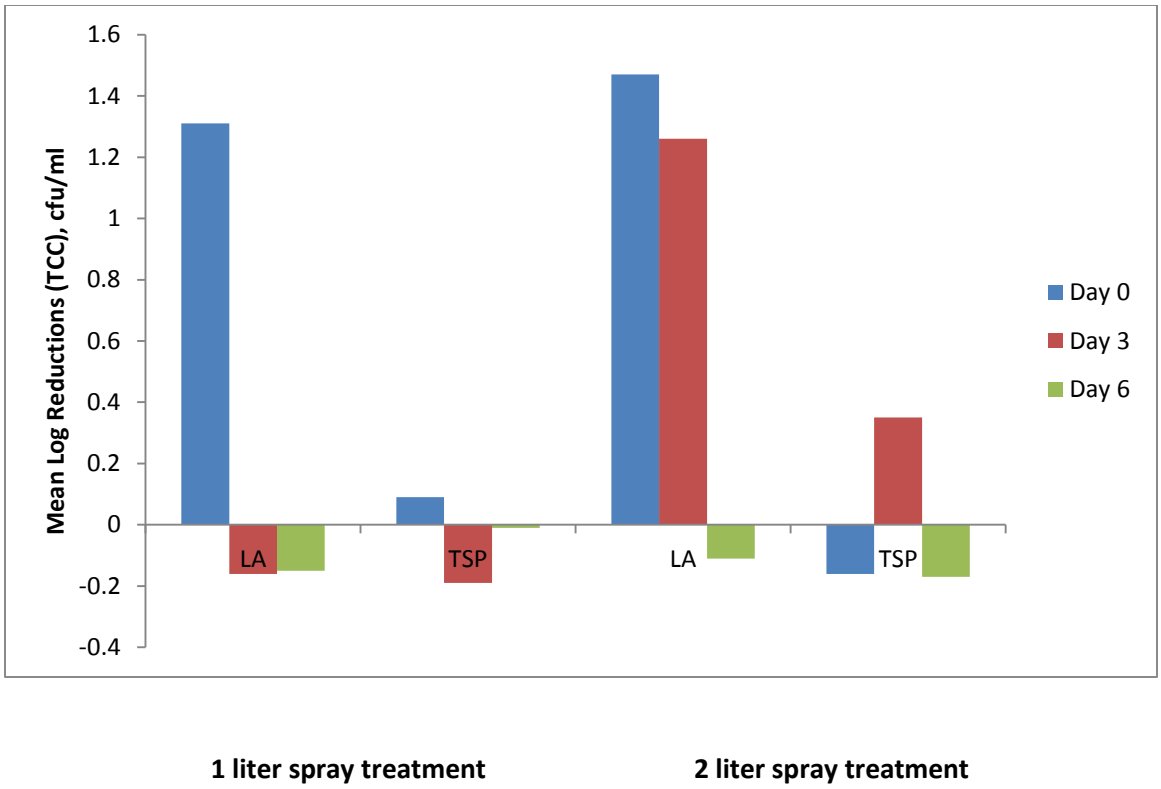


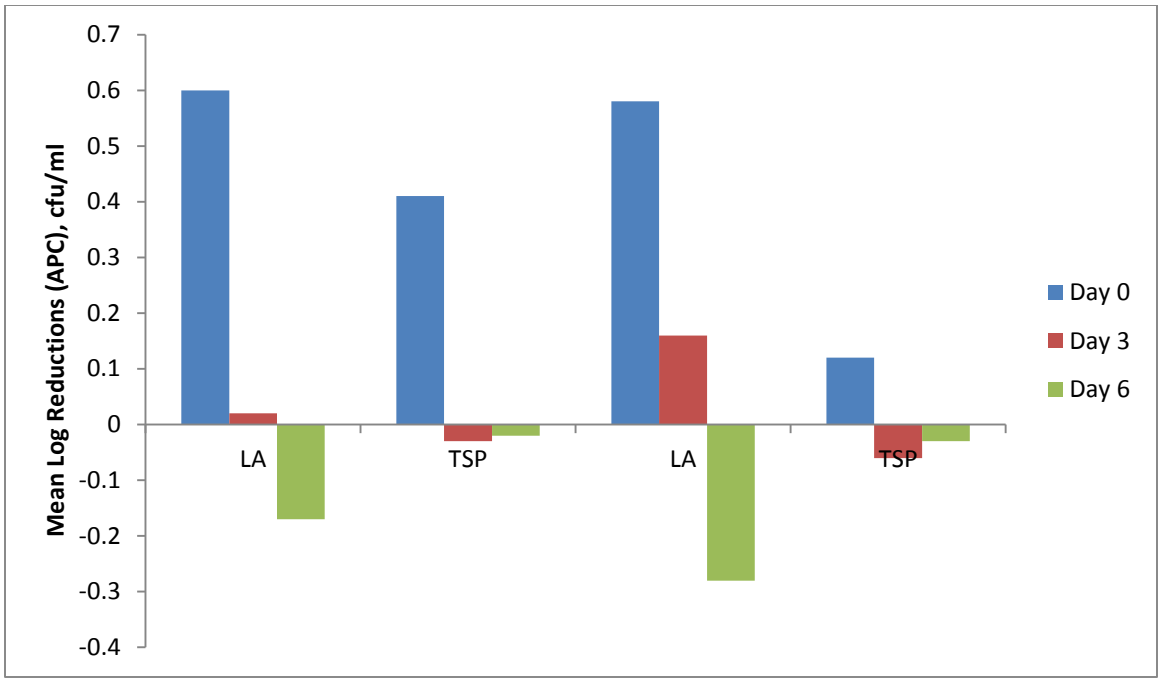

**Figure 4.4: The effects of the different acid and alkaline substances and the different modes of treatment on the bacteriological conditions CFU/ml (Total Coliform Count, TCC) on beef samples in Tudun-wada Market**

4.1.5 Mean log reductions of aerobic plate count for Sabo market

High reductions were seen on day 0 only at both volumes of the lactic acid treatment (1L and 2L). Reductions on microbial load were also observed on day 0 only of the TSP treatment with the 1L spray treatment giving better reductions than the 2L spray treatment. For all treatment {LA (1L), LA (2L), TSP (1L), TSP (2L)}, reduction effect on microbial load declined with increasing days of storage except on one occasion with LA treatment on day 0. Figure 4.5 gives better illustrations.

The multifactor analysis of variance on the $\log_{10}$ transformed values revealed no significant differences ($P>0.05$) between treatments, modes of treatment and their interactions. This implies that a combination of these factors were not significant means in reducing the aerobic microbial populations found on beef samples. By days of storage, the differences were significant meaning that the mean log reductions obtained varied with the days of storage.

There were no significant differences ($p>0.05$) revealed between various treatments; and between treatments and control (Appendix 9).




**1 liter spray treatment** **2 liter spray treatment**

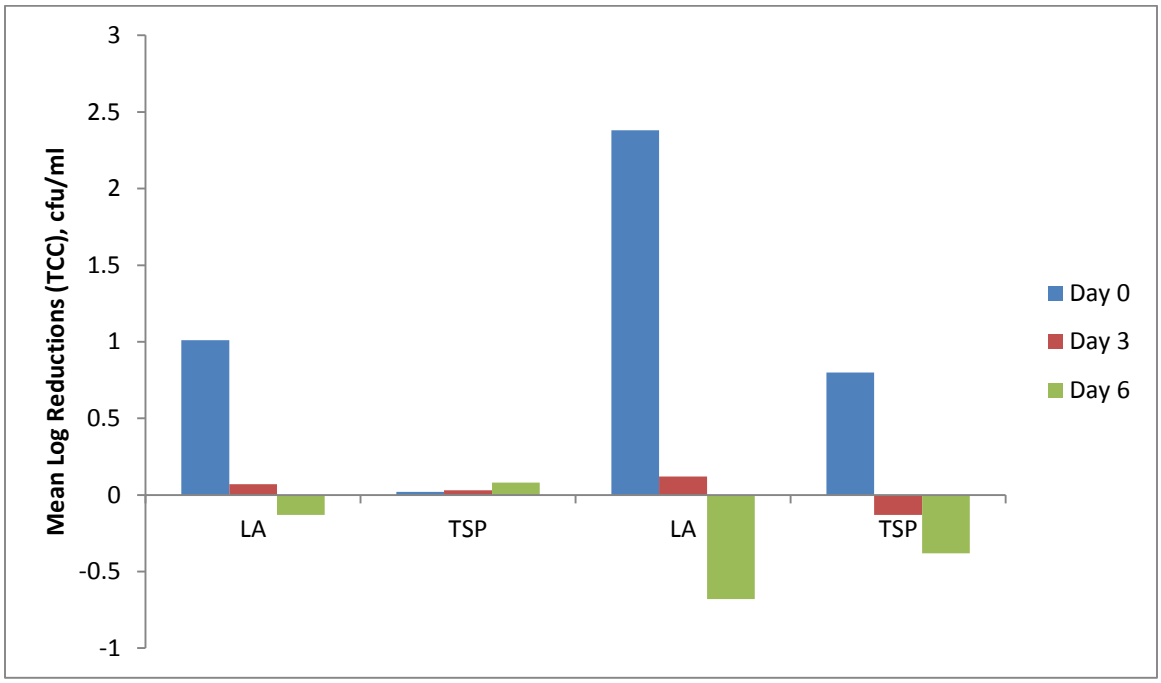
**Figure 4.5: The effects of the different acid and alkaline substances and the different modes of treatment on the bacteriological conditions CFU/ml(Aerobic Plate Count, APC) on beef samples in Sabo Market**

4.1.6 Mean log reductions of total coliform count for Sabo market

Apart from the TSP (1L) spray treatments which gave minimal reductions on microbial load with increasing days of storage, all other treatments {LA (1L), LA (2L), TSP (2L)} however declined in their reduction effects on microbial growth as the days of storage progressed (Figure 4.6).

The multifactor analysis of variance on the $\log_{10}$ transformed values revealed no significant differences ($P>0.05$) between treatments, modes of treatment and their interactions. This implies that a combination of these factors were not a significant means in reducing the microbial populations found on beef samples. By days of storage, the differences were significant meaning that the mean log reductions obtained varied with the days of storage.

There were no significant differences ($p>0.05$) observed between treatments {LA (1L) and LA (2L); LA (1L) and TSP (1L)}. The TSP (2L) treatment gave significant reductions ($p<0.05$) on day 0 only compared to the LA (2L) treatments. When compared to the control group, both modes of LA treatments generally gave more reductions on microbial load on days 0 and 3 of storage only. However, only the LA (2L) treatment recorded significant differences ($p<0.01$) than control (2L) on day 0 of storage (See appendix 6).




**1 liter spray treatment** **2 liter spray treatment**

**Figure 4.6: The effects of the different acid and alkaline substances and the different modes of treatment on the bacteriological conditions CFU/ml (Total coliform count, TCC) on beef samples in Sabon gari market**

**Table 4.1: Mean log reductions in aerobic plate counts (APC) and the total coliform counts (TCC) following different treatments with 1% lactic acid (LA) and 10% trisodium phosphate (TSP) at the three different markets (Samaru, Tudun-wada and Sabo) in Zaria Nigeria**

| Market | Mode of Treatment | Treatment | APC (Cfu/ml) | | | TCC (Cfu/ml) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Days of Storage | | | Days of Storage | | |
| | | | 0 | 3 | 6 | 0 | 3 | 6 |
| **Samaru** | 1L | LA | -0.64 | 1.18 | -0.23 | 0.25 | 0.90 | 0.28 |
| | | TSP | -2.11 | -0.44 | -0.45 | -0.19 | -0.71 | -1.33 |
| | 2L | LA | -0.59 | -0.43 | 0.42 | 0.36 | 0.42 | 0.43 |
| | | TSP | 0.23 | -1.65 | -0.55 | 1.34 | -0.57 | -1.29 |
| **Tudun-wada** | 1L | LA | 1.18 | 0.16 | 0.11 | 1.31 | -0.16 | -0.15 |
| | | TSP | 0.28 | 0.08 | -0.01 | 0.09 | -0.19 | -0.01 |
| | 2L | LA | 0.61 | 0.31 | 0.76 | 1.47 | 1.26 | -0.11 |
| | | TSP | 0.44 | 0.05 | -0.07 | -0.16 | 0.35 | -0.17 |
| **Sabo** | 1L | LA | 0.60 | 0.02 | -0.17 | 1.01 | 0.07 | -0.13 |
| | | TSP | 0.41 | -0.03 | -0.02 | 0.02 | 0.03 | 0.08 |
| | 2L | LA | 0.58 | 0.16 | -0.28 | 2.38 | 0.12 | -0.68 |
| | | TSP | 0.12 | -0.06 | -0.03 | 0.80 | -0.13 | -0.38 |

Mean log reduction= Mean log bacterial count (control) minus mean log bacterial count (treatment)

## 4.2 *E. coli* Isolation

Tables 4.2, 4.3 and 4.4 show the summary (in percentages) of the frequency of isolation of *E. coli* from beef samples bought from the three different markets (Samaru, Tudun-wada and Sabo respectively) following the different treatments on all the days of storage.

### 4.2.1 Samaru market

With treatment of meat carcasses with 1L spray treatment (LA), isolation rate of *E. coli* remained the same on all the days of storage at 8.3 %. The control group had an increase in the isolation rate of *E. coli* on the third day of storage (16.7 %) and afterwards declined to zero (0.0 %) on the sixth day. The 2 liter spray treatment for control and lactic acid had a similar pattern of isolation rate on all days of storage. For both, the isolation rate of *E. coli* increased on the third day of storage and then declined on the sixth day. With LA, a lower rate of *E. coli* isolation on all days of storage was recorded compared with the control. Both volumes of treatment with TSP had a similar effect on the isolation rate of *E. coli* on all days of storage. Beef samples treated with TSP had a high rate of *E. coli* isolation on day 0 and then consistently and progressively declined with increasing days of storage recording no growth (0.0 %) on the sixth day. There was however no significant difference ($p>0.05$) between different treatments on the frequency of isolation of *E. coli* in Samaru market. This is seen in Table 4.2.

**Table 4.2: Summary (in percentages) of the frequency of isolation of *E. coli* from beef samples bought from Samaru market following the different treatments on all the days of storage**

| Treatment Type | Mode of Treatment | No. of samples | Days of Storage (Percentages) | | |
|---|---|---|---|---|---|
| | | | 0 | 3 | 6 |
| **LA** | 1L | 12 | 8.30 | 8.30 | 8.30 |
| **CONTROL** | 1L | 12 | 8.30 | 16.70 | 0.00 |
| **LA** | 2L | 12 | 0.00 | 16.70 | 0.00 |
| **CONTROL** | 2L | 12 | 16.70 | 41.70 | 16.70 |
| **TSP** | 1L | 12 | 33.30 | 8.30 | 0.00 |
| **CONTROL** | 1L | 12 | 8.30 | 16.70 | 0.00 |
| **TSP** | 2L | 12 | 41.70 | 0.00 | 0.00 |
| **CONTROL** | 2L | 12 | 16.70 | 41.70 | 16.70 |

p=0.059

### 4.2.2 Tudun-wada market

Lactic acid (1 liter spray treatment) had relatively minimal reductions on the isolation rate of *E. coli* remaining the same (8.3 %) on days 0 and 3 of storage and then a complete reduction (0.0 %) on the sixth day of storage. The control group had less effect on the frequency of isolation of *E. coli*, increasing on day 3 of storage (16.7 %) and decreasing at the sixth day of storage (8.3 %) with a higher isolation rate than those treated with LA. The 2L spray treatment for LA and control had a similar pattern of effect. The rate of *E. coli* isolation increased on the third day of storage and then declined on the sixth day. Those treated with LA (2L) gave lower rates of *E. coli* isolation on all days of storage compared with the control group. Beef samples treated with TSP had a high rate of *E. coli* isolation on day 0 recording 50 % and 25 % for the 1L and 2L treatment respectively. This was followed by a sharp, progressive and consistent decline as the days of storage increased. There was significance observed ($P < 0.01$) between different treatments on the frequency of isolation of *E. coli* in Tudun-wada market.

**Table 4.3: Summary (in percentages) of the frequency of isolation of *E. coli* from beef samples bought from Tudun-wada market following the different treatments on all the days of storage**

| Treatment Type | Mode of Treatment | No. of Samples | Days of Storage (Percentages) | | |
|---|---|---|---|---|---|
| | | | 0 | 3 | 6 |
| **LA** | 1L | 12 | $8.30^{a}$ | $8.30^{b}$ | $0.00^{c}$ |
| **CONTROL** | 1L | 12 | $8.30^{a}$ | $16.70^{b}$ | $8.30^{c}$ |
| **LA** | 2L | 12 | $16.70^{a}$ | $25.00^{b}$ | $0.00^{c}$ |
| **CONTROL** | 2L | 12 | $25.00^{a}$ | $33.30^{b}$ | $0.00^{c}$ |
| **TSP** | 1L | 12 | $50.00^{a}$ | $16.70^{b}$ | $0.00^{c}$ |
| **CONTROL** | 1L | 12 | $8.30^{a}$ | $16.70^{b}$ | $8.30^{c}$ |
| **TSP** | 2L | 12 | $25.00^{a}$ | $16.70^{b}$ | $8.30^{c}$ |
| **CONTROL** | 2L | 12 | $25.00^{a}$ | $33.30^{b}$ | $0.00^{c}$ |

$p= 0.0031$; $^{a,b,c}$: Letters with different superscripts are statistically significant

### 4.2.3 Sabo market

Beef samples treated with LA at both volumes (1L and 2L) had increase in the isolation rate of *E. coli* on the third day of storage (16.7 %) and then recorded a complete reduction on the sixth day (0.0 %). For the LA (1L) treatment, reduction on *E. coli* isolation was recorded only on the sixth day of storage compared with the control (1L). The 2L treatment with LA however gave better reductions on *E. coli* isolation than the control (2L) on all days of storage. Although TSP treatment (1L) recorded higher *E. coli* isolation than the control on days 0 and 3 of storage, there was however a consistent and progressive decline recording no *E. coli* growth (0.0 %) on the sixth day of storage. With the 2L treatment, observations showed that the control group gave better reductions than the TSP-treated group on the sixth day of storage (table 4.4). There was no significant difference ($p>0.05$) between different treatments on the frequency of isolation of *E. coli* in Sabo market. This is further illustrated in table 4.4.

**Table 4.4: Summary (in percentages) of the frequency of isolation of *E. coli* from beef samples bought from Sabo market following the different treatments on all the days of storage**

| Treatment | Mode of Treatment | No. of Samples | Days of Storage (Percentages) | | |
|---|---|---|---|---|---|
| | | | 0 | 3 | 6 |
| **LA** | 1L | 12 | 8.30 | 16.70 | 0.00 |
| **CONTROL** | 1L | 12 | 8.30 | 8.30 | 16.70 |
| **LA** | 2L | 12 | 0.00 | 16.70 | 0.00 |
| **CONTROL** | 2L | 12 | 33.30 | 33.30 | 8.30 |
| **TSP** | 1L | 12 | 41.70 | 25.00 | 0.00 |
| **CONTROL** | 1L | 12 | 8.30 | 8.30 | 16.70 |
| **TSP** | 2L | 12 | 33.30 | 33.30 | 25.00 |
| **CONTROL** | 2L | 12 | 33.30 | 33.30 | 8.30 |

### 4.2.4 *E. coli* isolation on both volumes of treatment (1L and 2L) and control for all markets (Samaru, Tudun –wada and Sabo Market).

Table 4.5 gives an overview of *E. coli* isolation on both volumes of treatment (1L and 2L) and control for the three markets following treatments per days of storage. For all markets observed, an overview of *E. coli* isolation on both volumes of treatment (1L and 2L) and control reported that meat samples treated with LA and control generally increased on the third day of storage and then declined on the sixth day. However, those treated with LA generally gave lower *E. coli* isolations than the control group on all days of storage [with the exception of Samaru market which had a similar prevalence of *E. coli* on the sixth day of storage with that of the control (8.3 %)]. *E. coli* on meat samples treated with TSP had higher rates of isolation on day 0 of storage with a consistent and sharp decline on days 3 and 6 respectively. These are further illustrated in figures 4.7, 4.8 and 4.9.

**Table 4.5: An overview of *E. coli* isolation (in percentages) on both volumes of treatment and control (1L and 2L) for the three markets (Samaru, Tudun-wada and Sabo) following treatments per days of storage**

| Market | Treatment Type | No. of Samples | Days of Storage (Percentages) | | |
|---|---|---|---|---|---|
| | | | 0 | 3 | 6 |
| **Samaru** | LA | 24 | 4.20 | 13.00 | 8.30 |
| | TSP | 24 | 37.50 | 4.17 | 0.00 |
| | CONTROL | 24 | 13.00 | 29.20 | 8.30 |
| **Tudun-wada** | LA | 24 | 13.00 | 17.00 | 0.00 |
| | TSP | 24 | 37.50 | 17.00 | 4.20 |
| | CONTROL | 24 | 17.00 | 25.00 | 4.20 |
| **Sabo** | LA | 24 | 4.17 | 17.00 | 0.00 |
| | TSP | 24 | 37.50 | 29.17 | 13.00 |
| | CONTROL | 24 | 20.80 | 20.80 | 13.00 |

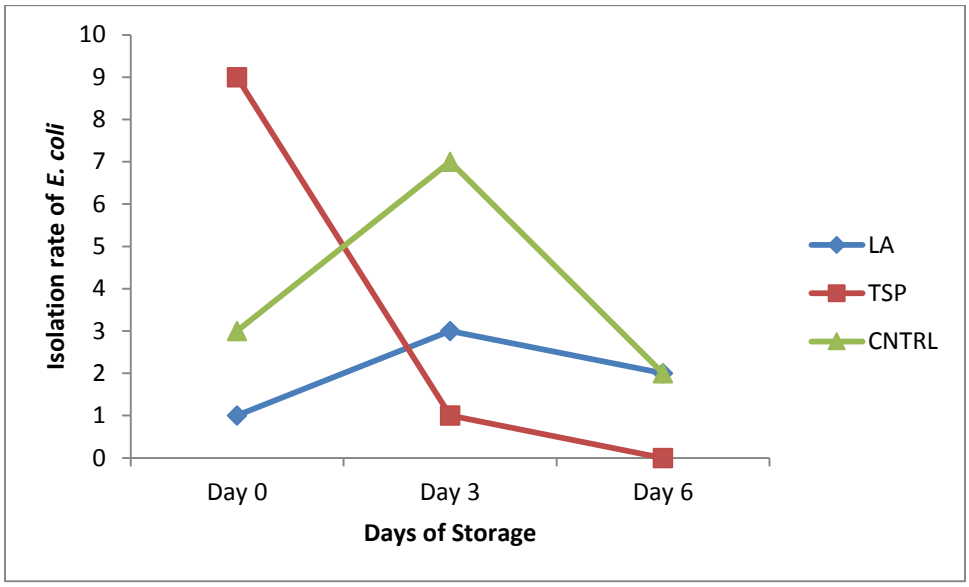



**Figure 4.7: An overview of *E. coli* isolation on both volumes of treatment (1L and 2L) and control for Samaru market following treatments per days of storage**

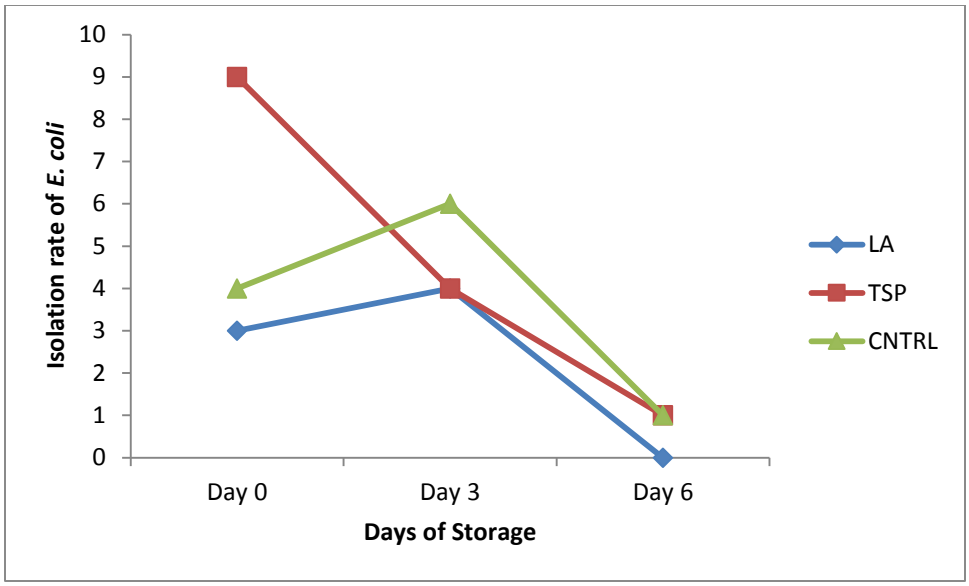



**Figure 4.8: An overview of *E. coli* isolation on both volumes of treatment (1L and 2L) and control for Tudun-wada market following treatments per days of storage**

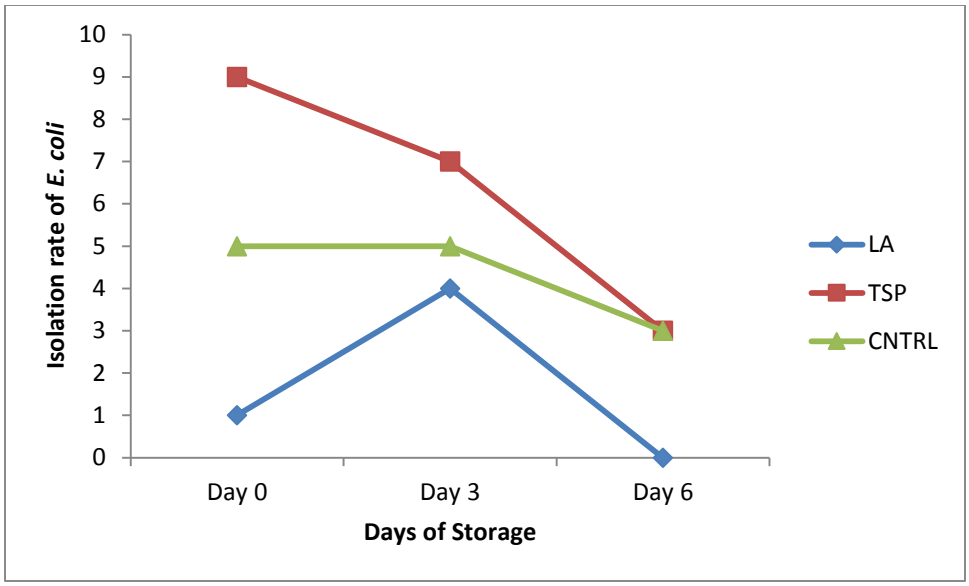



**Figure 4.9: An overview of *E. coli* isolation on both volumes of treatment (1L and 2L) and control for Sabo market following treatments per days of storage**

## 4.3 Sensory Evaluation of Raw and Roasted Beef Samples

Table 4.6 shows the preference result evaluated by the panelists for raw (appearance and smell) and roasted beef samples (appearance, smell and taste) for the treated (Lactic acid) and control group.

### 4.3.1 Sensory evaluation of raw beef samples

Lactic acid was used as the decontaminant for sensory evaluations since they generally gave more potency in reducing microbial load on beef samples than the TSP treatment. From the sensory analyses results evaluated by the seven member-panels, 4 panelists showed preference for the untreated (control) raw beef samples based on appearance and smell while two (2) preferred the lactic acid-treated samples. One (1) panelist had no special preference for either (Control or treated) and would willingly utilize any of them in preparing meals irrespective of whether they are treated or not, judging by appearance or smell.

### 4.3.2 Sensory evaluation of roasted beef samples

For the evaluation of the roasted beef samples, the situation was different. While one (1) panelist preferred the untreated (control) beef samples in terms of appearance, smell and taste, 4 opted for the lactic acid-treated beef samples judging by the same characteristics. Two (2) panelists had no special preference for any and would willingly utilize any to prepare dishes and meals judging by the appearance, smell and taste.

**Table 4.6: Preference shown by the panelists for raw and roasted beef samples, treated (Lactic acid) and control**

| Sensory Characteristic | Treatment | No of Panelist showing Preference (%) (n=7) | |
|---|---|---|---|
| | | Raw | Roasted |
| Appearance and Smell | Control | 4 (57) | 1(14) |
| | Lactic acid | 2 (29) | 4 (57) |
| | No special preference | 1(14) | 2 (29) |
| Taste | Control | - | 1(14) |
| | Lactic acid | - | 4 (57) |
| | No special preference | - | 2 (29) |

Note: Score is based simply on the number of panelists showing preference out of the 7 panelists.

# CHAPTER FIVE

## 5.0 DISCUSSION

The retail meat market is one of the supply chains of the beef industry since it is a source where most consumers purchase their meat. In this study, an investigation was carried out on the effect of Lactic acid (LA) and Trisodium phosphate (TSP) treatment on the shelf-life of beef sold in retail markets at the Zaria metropolis.

The findings in this study showed that decontamination with acid and alkaline substances did not give significant improvement on the bacteriological condition of beef samples purchased from retail markets in the Zaria metropolis.

Beef, a culinary name of bovine species especially cattle has an ideal medium and favourable chemical composition which exposes it to contamination by spoilage and pathogenic bacteria. The microflora of meat is determined by microbial contamination during processing on the slaughter line, transportation, poor handling, marketing and hygienic status which form resident microbiota that will potentially contribute to the initial microbial load on beef before storage starts (De Filipis *et al*., 2013).

Colony count is a measure of the microbial quality of the meat and according to the Microbiological guidelines for food (2014), values for raw meat were attained at $10^6$ for satisfactory, $10^6$- $10^7$ for borderline and $\geq 10^7$ for un-satisfactory meat. From all markets sampled (Samaru, Tudun-wada and Sabo), observations showed heavy bacteriological load carried by beef samples with counts for both APC (Aerobic plate count) and TCC (Total coliform counts) ranging from $10^6$ to $10^9$ cfu/ml. Amongst these studied microbial categories, the population counts in control as well as in treated beef samples were higher for APC than in TCC. This is in

line with the work of other authors who compared the distribution of the APCs and TCCs on the natural microflora of beef samples sold in retail markets (Lawan *et al*., 2011; Ukut *et al*., 2010). The high microbial counts may be attributed to the manner in which the meat is being processed, transported and handled as well as the cross-contamination that may have arisen due to contacts with equipment and dirty surfaces at retail points. The attachment of potential spoilage and pathogenic bacteria to food contact surfaces and the subsequent biofilm formation has been seen to present serious challenges to the meat (Giaouris *et al.*, 2014).

Results from the decontaminants gave inconsistent patterns in the reduction effects on the bacterial populations with the different volumes of acid and alkaline substances used. Although, LA treatments were generally more potent in reducing microbial load than TSP treatments, there were generally no significant differences between the LA treatments and the control. Significant differences for lactic acid were recorded only at day 0 of storage with both the LA (1L) and LA (2L) spray treatment for Tudun-wada and Sabo market respectively when compared with the control. These patterns are further illustrated in the bar charts which picture the mean log reductions for the different treatments with respect to markets and microbial load. The higher the columns on the bar charts, the greater the mean log reductions achieved. Results based on the interactions between factors (treatment, mode of treatment and days of storage) showed that interactions were observed with the TCC of Samaru markets. All other interactions between factors with respect to microbial load and markets were not significant as they were not effective in causing reductions on the microbial load of beef samples obtained from retail markets.

Results showing the potency of LA over TSP have been reported in various studies (Bin Jasass, 2008; Mohammed and Sallam, 2009). This may be due to the destructive effect of lactic acid on the proteolytic bacteria particularly Gram-negative bacteria through induction of low pH and

liberation of undissociated acid molecules that change the permeability of the microbial cell membrane (Mohammed and Sallam, 2009). Also, Bautista *et al*. (1997) in an attempt to determine the efficacy of antimicrobial rinses on turkey carcasses using response surface designs reported that Lactic acid had some effect against *Salmonella* spp whereas TSP irrespective of concentration and pressure were not effective against *Salmonella* spp. However, lactic acid in some cases was shown to be ineffective on the shelf-life of meat carcasses in a commercial processing environment with concentrations even as high as 2% and 5% (Harris *et al*., 2012). Also, in a study to ascertain the improvement of shelf-life of buffalo meat during retail display using lactic acid, clove oil and vitamin C, Naveena *et al* (2006) discovered that the difference in results of the lactic acid treatment on the meat were insignificant compared to the control.

Various studies have however reported the efficacy of these decontaminants (Mohammed and Sallam, 2009; Okolocha and Ellerbroek, 2005; Rio *et al*., 2007). It is imperative to note that the mean log reductions reported by most workers on the effect of acid and alkaline treatments on the bacteriological conditions of meat were carried out in slaughter houses with still warm carcasses and not on retail markets. The microbial load on meat has been reported to increase with increase in time. This is to say that the effect of time on the level of microbial load on beef can be seen to be a significant factor on meat purchases at retail markets (Lawan *et al*., 2011). Keeping meat at ambient temperature for a long time which is commonly done by meat retailers in the market may allow multiplication of pathogenic bacteria (Umoh *et al*., 2002). Also, the level of contamination of meat is said to have a higher value of microbial load at the meat market when compared to what was obtained at slaughter points due to the various levels of handling, transportation and unhygienic practices that the meat is subjected to (Bhandare *et al*., 2007). The presence of a high number of bacterial counts increases the chances of meat spoilage in a short

time, thus making the decontaminant less effective on such meat of high microbial load using the same volume and concentration that are applied at slaughter houses. The afore- mentioned reasons may be why the effect of decontaminants at slaughter houses show more efficacy as shown by these authors  than what was reported in this study on meat purchased from retail markets. In addition, bacteria have been seen to form extracellular binding polymers on the surface of meat, thereby effectively anchoring the bacteria to the surface forming stronger permanent bonds with the meat. Therefore, the ability of bacteria to penetrate meat tissues could be a contributory factor as this has significant implications for the effectiveness of a decontamination process, as these cells will be protected from the effect of sanitizing chemicals (Fratamico *et al*., 1996).

From the results of the mean logs of the total aerobic count and total coliform count on beef samples, some observations recorded a more reductive effect on the meat microbial load for the control group than the treated group (Appendix 4 to 8). Although, LA treatments were generally more potent than the TSP, there were however some recordings of the control performing better than the LA treatment on some days of storage with different markets (See Appendix 4 to 8). Similar findings have been reported in other studies (Brackett *et al*., 1994; El- Khatteib et al., 1993; Fratamico *et al.,* 1996, Harris *et al*., 2012). This ineffectiveness of lactic acid treatment to reduce microbial load as observed from some of the results recorded may be due to higher acid tolerance of some pathogens and increase of pathogenic growth due to possible reduction of microbial competition. (Beyaz and Tayar, 2010). In addition, exposure to low pH and organic acids in the bovine intestinal tract may also result in the induced acid resistance of *E. coli* and other pathogens that may subsequently contaminate beef samples. (Berry and Cutter, 2000)

Isolation of *E. coli* from beef sampled in all the three different markets was also used to assess for shelf-life of beef as *E. coli* are bio-marker organisms used in many food control laboratories to assess the meat with respect to its microbiological safety by the estimation of the growth of *E. coli* (Enne de boer, 1998; Greer *et al*., 1994). A unique trend of pattern was observed for all the three markets with respect to the effect of these decontaminants on the frequency of isolation and growth of *E. coli* on the beef samples. Beef samples following treatment with TSP had a high isolation rate of *E. coli* on day 0 of storage. This was however followed by a sharp and consistent decline as the days of storage progressed.

Beef samples sprayed with LA and water (control) had a similar pattern of effect on the frequency of isolation of *E. coli* as the days of storage progressed. Both had lower frequency of isolation on day 0 of storage followed by an increase on day 3 and then a decrease on the sixth day of storage. This high frequency of *E. coli* isolation on the third day of storage may be due to the increase in the population growth rate (log phase) of the *E. coli* cell by its nutrient utilization of the meat substrate. This however declines on the sixth day of storage which may be as a result of the build-up of toxic metabolites as the meat product ages (with increasing days of storage) and also due to intraspecific competition for nutrients. However, for all markets and with all beef samples, LA treatment gave lower rates of *E. coli* isolation for all the days of storage compared with the control. In other words, *E. coli* growth rates were lower on beef samples sprayed with lactic acid compared to those sprayed with water. The lower rate of *E. coli* isolation on beef samples treated with LA than the corresponding control group could be due to the residual effect of LA treatment on the microorganism during the storage (Martinez *et al*., 2002; Ramirez *et al*., 2001). However, significant differences ($p<0.01$) were only recorded with samples from Tudun-wada market. The high rate of *E. coli* isolation on beef samples treated with TSP on day 0 of

storage tallies with what was carried out by other researchers. Bautista *et al*. (1997) reported that TSP treatment had no effect on the growth of *Salmonella* spp irrespective of the concentration and pressure used. Okolocha and Ellerbroek (2005) in their study to determine the influence of acid and alkaline treatment on the shelf-life of poultry meat discovered that carcasses treated with TSP by dipping gave higher prevalence of *Salmonella* spp than even the control (water). This higher rate of *E. coli* isolation on day 0 of storage on beef samples treated with TSP may be due to the fact that under aerobic conditions, the abundance of a variety of bacterial cytoplasmic proteins is increased by high pH. These cytoplasmic proteins are necessary for growth and support the growth of the bacterial cell. In addition, the production of several enzymes (deaminases) also increases at an alkaline pH and these provide metabolic adaptations to the alkaline challenge by lowering the cytoplasmic pH of the cell (Padan *et al*., 2005). However, reduction of *E. coli* prevalence with increasing days of storage as also observed with TSP treatment may be due to the destructive effect of refrigeration on an already stressed cell.

The results obtained from the sensory evaluations showed that the organoleptic properties of the beef samples were not affected by the treatment (LA) indicating that the level at which it is applied (1 %) posed no threat to the aesthetic sensibilities of the consumers. For the evaluations of the raw beef samples, the higher preferences recorded by the panelists for beef samples sprayed with water (control) identifies with the work of Stivarius *et al.* (2002b) who recorded that lactic acid-treated beef trimmings reduced ground beef redness. Also, Pipek *et al*, (2005) recorded that meat redness decreased slightly after treatment with steam and lactic acid. This may be due to the effect of a lower pH caused by the lactic acid on the meat surface which promotes pigment oxidation thereby decreasing redness and oxymyoglobin content (Stivarius *et al.* 2002a). Two (2) of the panelists however recorded preference for lactic acid-treated samples

while one (1) showed no special preference indicating that non-treated samples alongside treated can both be used in preparing meals without affecting the aesthetic sensibilities of the consumer in terms of its appearance and smell.

For the evaluations of roasted beef samples, higher preferences were recorded for lactic acid-treated samples. Studies that showed that lactic acid-treated samples did not affect sensory quality of meat (chicken or beef) have been recorded (Deumier, 2006; Okolocha and Ellerbroek, 2005; Smulders and Greer, 1998). This explains the fact that lactic acid as a decontaminant does not affect the aesthetic sensibilities of the consumer and are generally without effect on the desirable sensory properties of meat and therefore can be used in preparing meals.

# CHAPTER SIX

# 6.0 CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

Comparing the effects of the treatments on the microbial load of beef samples, the microbial reductions throughout storage either increased, decreased or did not vary, depending on the microbial group and decontaminants/chemicals involved. Although, Lactic acid treatment gave better reductions on microbial load than Trisodium phosphate treatment, they were not significantly different from the control. Lactic acid treatments only recorded significant reductions with the 1L (APC) and 2L (TCC) treatments for Tudun-wada and Sabo market respectively as opposed to the control on day 0 of storage. Mode (volume) of treatment did not significantly affect the microbial load of beef samples purchased from the retail markets.

The effect of the decontaminants on the isolation rate of *E. coli* indicated that lactic acid was effective against *E. coli* species when compared to the control. Lactic acid had 4.2%, 13%, 8.3% compared to control with 13%, 29.2%, 8.3% on days 0, 3 and 6 respectively for Samaru market. Tudun-wada market recorded 13%, 17%, 0% with LA compared to 17%, 25%, 4.2% (control) on days 0, 3 and 6 of storage respectively. Sabo market recorded 4.17%, 17%, 0% with LA compared to control having 20.8%, 20.8%, 13% on days 0, 3 and 6 of storage respectively. Although, TSP treatment reduced *E. coli* consistently, the frequency of isolation were generally higher (37.5% for all markets) than the control group (13%, 17%, 20% for Samaru, Tudun-wada and Sabo market respectively) on day 0 of storage. TSP also recorded higher rates of isolation of *E. coli* (37.5% for all markets) than the lactic acid treated group (4.2%, 13%, 4.17%) on day 0 of storage.

Lactic acid treatment did not affect the organoleptic properties of roasted beef samples as preference for its apperarance, smell and taste was recorded by 57% of the panelists compared to 14% recorded for control. For raw beef samples, there was a higher preference for the control group (57%) than those treated with LA (29%) in terms of its appearance and smell.

Overall, the findings of this study reveal that the influence of lactic acid and TSP were not overly effective on the shelf-life of beef samples sold in retail markets in Zaria metropolis. Lactic acid treatments however showed reduction effect on the isolation rate of *E. coli* as opposed to the control and they do not affect the organoleptic properties of beef samples.

## 6.2 Recommendations

In efforts to meet regulatory requirements and ultimately provide safe products to consumers, a variety of interventions with the objective of reducing contamination on beef and meat products should be taken.

I. Meat handlers/processors should observe strict hygienic measures so that they may not serve as sources of chance inoculation of microorganisms and faecal contamination of fresh meat and other meat products.
II. Decontamination processes using lactic acid and trisodium phosphate to reduce or eliminate the microbial load on beef should be employed at the abattoir or under industrial conditions to ensure its proper effectiveness.
III. Sanitary authorities should ensure and enforce strict compliance to the recommended food standards as regards the processing and sales of fresh and packaged meat products.

IV. Meat handlers and sellers should be educated on the adverse effect of lack of proper personal hygiene and sanitation.

V. In addition, good processing practices such as observing strict hygienic measures during butchering should be adhered to by butchers and those selling meat.

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## APPENDICES

### Appendix I: Differences Comparing the TCC of the Three Different Markets

| Treatment | N | Day | Samaru | Tudun-Wada | Sabo |
|---|---|---|---|---|---|
| LA 1L | 12 | 0 | 6.54±0.16 | 5.59±0.89 | 5.74±0.81 |
| | 12 | 3 | 6.52±0.39 | 8.36±0.19 | 8.69±0.04 |
| | 12 | 6 | 6.95±0.20 | 8.48±0.06 | 8.46±0.17 |
| LA 2L | 12 | 0 | 6.49±0.41 | 5.76±0.84 | 5.12±0.70 |
| | 12 | 3 | 6.87±0.18 | 7.62±0.50 | 8.49±0.21 |
| | 12 | 6 | 6.96±0.21 | 8.26±0.15 | 8.43±0.14 |
| TSP 1L | 12 | 0 | 6.98±0.79 | 6.81±0.76 | 6.74±0.58 |
| | 12 | 3 | 8.13±0.30 | 8.40±0.54 | 8.73±0.13 |
| | 12 | 6 | 8.56±0.27 | 8.34±0.17 | 8.25±0.16 |
| TSP 2L | 12 | 0 | 5.51±0.88 | 7.39±0.68 | 6.70±0.73 |
| | 12 | 3 | 7.86±0.53 | 8.53±0.24 | 8.73±0.07 |
| | 12 | 6 | 8.68±0.16 | 8.32±0.10 | 8.14±0.13 |
| CNTRL 1L | 12 | 0 | 6.79±0.16 | 6.90±0.78 | 6.75±0.75 |
| | 12 | 3 | 7.42±0.03 | 8.20±0.25 | 8.76±0.04 |
| | 12 | 6 | 7.23±0.10 | 8.33±0.20 | 8.33±0.18 |
| CNTRL 2L | 12 | 0 | 6.85±0.22 | 7.23±0.69 | 7.50±0.69 |
| | 12 | 3 | 7.30±0.09 | 8.88±0.18 | 8.60±0.09 |
| | 12 | 6 | 7.38±0.04 | 8.15±0.07 | 7.75±0.50 |

**Appendix II: Differences Comparing the APC of the Three Different Markets**

| Treatment | N | Day | Samaru | Tudun-Wada | Sabo |
|---|---|---|---|---|---|
| LA 1L | 12 | 0 | 7.70±0.50 | 7.39±0.84 | 8.02±0.55 |
| | 12 | 3 | 7.34±0.66 | 8.78±0.18 | 8.83±0.05 |
| | 12 | 6 | 8.64±0.18 | 8.77±0.14 | 8.78±0.09 |
| LA 2L | 12 | 0 | 8.04±0.55 | 8.48±0.31 | 7.97±0.55 |
| | 12 | 3 | 7.92±0.50 | 8.70±0.24 | 8.60±0.18 |
| | 12 | 6 | 8.12±0.17 | 8.16±0.53 | 8.84±0.12 |
| TSP 1L | 12 | 0 | 9.18±0.15 | 8.29±0.26 | 8.22±0.56 |
| | 12 | 3 | 8.96±0.20 | 8.86±0.15 | 8.88±0.08 |
| | 12 | 6 | 8.85±0.17 | 8.90±0.15 | 8.63±0.09 |
| TSP 2L | 12 | 0 | 7.22±0.80 | 8.64±0.21 | 8.43±0.58 |
| | 12 | 3 | 9.15±0.13 | 8.96±0.14 | 8.81±0.07 |
| | 12 | 6 | 9.09±0.10 | 8.99±0.16 | 8.59±0.10 |
| CNTRL 1L | 12 | 0 | 7.06±0.62 | 8.57±0.57 | 8.62±0.26 |
| | 12 | 3 | 8.51±0.18 | 8.94±0.18 | 8.85±0.03 |
| | 12 | 6 | 8.40±0.17 | 8.88±0.16 | 8.61±0.12 |
| CNTRL 2L | 12 | 0 | 7.45±0.74 | 9.09±0.15 | 8.55±0.28 |
| | 12 | 3 | 7.50±0.46 | 9.01±0.10 | 8.75±0.05 |
| | 12 | 6 | 8.54±0.22 | 8.91±0.15 | 8.56±0.11 |

## Appendix III: Sensory Analyses Form

**I Evaluation of Fresh Beef Carcasses**

1. Is there any difference in the appearance of the two groups (treated and not treated) of samples presented in a triangle test? Two of the three carcasses belong to one group, one to another group. (Please tick one)

Yes No

2. Is there any noticeable smell (odour) from any of the samples presented?

Yes No

If Yes, which one of the samples?

3. Among the two groups of samples presented which two within each group do you think belong together? (Please tick the numbers bearing the sample numbers that belong together).

Group A: 1 2 3

Group B: 1 2 3

Which of the sample in each group do you prefer?

Why do you prefer the samples?

_____

**II Evaluation of Roasted Beef Carcasses**

1 Is there any difference in the appearance of the two groups (treated and not treated) of samples presented in a triangle test? Two of the 3 carcasses belong to one group, one to another group. (Please tick one)

Yes No

2 Is there any noticeable smell (odour) from any of the samples presented?

Yes No

If Yes, which of the samples?

3 Among the samples presented, which two do you think belongs together? (Please tick the two boxes bearing the sample numbers that belong together.)

1 2 3 4. None

Which do you prefer?

Why do you prefer the samples?

_____

**III Evaluation of Cut Pieces**

1 Among the two groups of cut beef pieces, which do you prefer?

1 2

Why do you prefer the chosen group? (Please briefly write down in one or two words the reason for your choice under the following attributes)

A Appearance _____

B Smell _____

C Taste _____

## Appendix IV: Statistical Analyses for the Total Coliform Count (TCC) of Samaru market

LA1L vs LA2L

| Days of storage | LA 1L | LA 2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.544 | 6.494 | -0.050 | 0.097 | P > 0.05 | ns |
| Day 3 | 6.518 | 6.871 | 0.353 | 0.682 | P > 0.05 | ns |
| Day 6 | 6.948 | 6.957 | 0.008 | 0.016 | P > 0.05 | ns |

LA1L vs TSP1L

| Days of storage | LA 1L | TSP 1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.544 | 6.978 | 0.434 | 0.838 | P > 0.05 | ns |
| Day 3 | 6.518 | 8.125 | 1.608 | 3.104 | P<0.01 | ** |
| Day 6 | 6.948 | 8.560 | 1.612 | 3.112 | P<0.01 | ** |

LA1L vs CNTRL1L

| Days of storage | LA 1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.544 | 6.792 | 0.248 | 0.478 | P > 0.05 | ns |
| Day 3 | 6.518 | 7.416 | 0.898 | 1.735 | P > 0.05 | ns |
| Day 6 | 6.948 | 7.228 | 0.280 | 0.541 | P > 0.05 | ns |

LA2L vs TSP2L

| Days of storage | LA2L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.494 | 5.508 | -0.986 | 1.903 | P > 0.05 | ns |
| Day 3 | 6.871 | 7.863 | 0.992 | 1.915 | P > 0.05 | ns |
| Day 6 | 6.957 | 8.675 | 1.718 | 3.318 | P<0.01 | ** |

LA2L vs CNTRL2L

| Days of storage | LA2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.494 | 6.853 | 0.358 | 0.692 | P > 0.05 | ns |
| Day 3 | 6.871 | 7.295 | 0.424 | 0.819 | P > 0.05 | ns |
| Day 6 | 6.957 | 7.382 | 0.425 | 0.821 | P > 0.05 | ns |

TSP1L vs TSP2L

| Days of storage | TSP1L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.978 | 5.508 | -1.470 | 2.838 | P < 0.05 | * |
| Day 3 | 8.125 | 7.863 | -0.263 | 0.507 | P > 0.05 | ns |
| Day 6 | 8.560 | 8.675 | 0.115 | 0.222 | P > 0.05 | ns |

TSP1L vs CNTRL 1L

| Days of storage | TSP1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.978 | 6.792 | -0.187 | 0.361 | P > 0.05 | ns |
| Day 3 | 8.125 | 7.416 | -0.709 | 1.369 | P > 0.05 | ns |
| Day 6 | 8.560 | 7.228 | -1.332 | 2.571 | P < 0.05 | * |

TSP2L vs CNTRL 2L

| Days of storage | TSP2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 5.508 | 6.853 | 1.344 | 2.595 | P < 0.05 | * |
| Day 3 | 7.863 | 7.295 | -0.568 | 1.096 | P > 0.05 | ns |
| Day 6 | 8.675 | 7.382 | -1.293 | 2.497 | P < 0.05 | * |

**Appendix V: Statistical Analyses for the Total Coliform Count (TCC) of Tudun-wada market**

| LA1L vs LA2L | | | | | | |
|---|---|---|---|---|---|---|
| Days of storage | LA 1L | LA 2L | Difference | t | P value | Summary |
| Day 0 | 5.591 | 5.760 | 0.169 | 0.240 | P > 0.05 | ns |
| Day 3 | 8.363 | 7.620 | -0.743 | 1.053 | P > 0.05 | ns |
| Day 6 | 8.478 | 8.260 | -0.218 | 0.309 | P > 0.05 | ns |
| | | | | | | |
| LA1L vs TSP1L | | | | | | |
| Days of storage | LA 1L | TSP 1L | Difference | t | P value | Summary |
| Day 0 | 5.591 | 6.813 | 1.222 | 1.733 | P > 0.05 | ns |
| Day 3 | 8.363 | 8.395 | 0.033 | 0.046 | P > 0.05 | ns |
| Day 6 | 8.478 | 8.343 | -0.135 | 0.192 | P > 0.05 | ns |
| | | | | | | |
| LA1L vs CNTRL1L | | | | | | |
| Days of storage | LA 1L | CNTRL1L | Difference | t | P value | Summary |
| Day 0 | 5.591 | 6.902 | 1.311 | 1.859 | P > 0.05 | ns |
| Day 3 | 8.363 | 8.201 | -0.162 | 0.229 | P > 0.05 | ns |
| Day 6 | 8.478 | 8.330 | -0.148 | 0.209 | P > 0.05 | ns |
| | | | | | | |
| LA2L vs TSP2L | | | | | | |
| Days of storage | LA2L | TSP2L | Difference | t | P value | Summary |
| Day 0 | 5.760 | 7.391 | 1.631 | 2.313 | P > 0.05 | ns |
| Day 3 | 7.620 | 8.533 | 0.913 | 1.295 | P > 0.05 | ns |
| Day 6 | 8.260 | 8.319 | 0.059 | 0.084 | P > 0.05 | ns |
| | | | | | | |
| LA2L vs CNTRL2L | | | | | | |
| Days of storage | LA2L | CNTRL2L | Difference | t | P value | Summary |
| Day 0 | 5.760 | 7.229 | 1.469 | 2.084 | P > 0.05 | ns |
| Day 3 | 7.620 | 8.883 | 1.263 | 1.791 | P > 0.05 | ns |
| Day 6 | 8.260 | 8.151 | -0.109 | 0.155 | P > 0.05 | ns |
| | | | | | | |
| TSP1L vs TSP2L | | | | | | |
| Days of storage | TSP1L | TSP2L | Difference | t | P value | Summary |
| Day 0 | 6.813 | 7.391 | 0.578 | 0.820 | P > 0.05 | ns |
| Day 3 | 8.395 | 8.533 | 0.138 | 0.196 | P > 0.05 | ns |
| Day 6 | 8.343 | 8.319 | -0.023 | 0.033 | P > 0.05 | ns |
| | | | | | | |
| TSP1L vs CNTRL 1L | | | | | | |
| Days of storage | TSP1L | CNTRL1L | Difference | t | P value | Summary |
| Day 0 | 6.813 | 6.902 | 0.089 | 0.127 | P > 0.05 | ns |
| Day 3 | 8.395 | 8.201 | -0.194 | 0.275 | P > 0.05 | ns |
| Day 6 | 8.343 | 8.330 | -0.013 | 0.018 | P > 0.05 | ns |
| | | | | | | |
| TSP2L vs CNTRL 2L | | | | | | |
| Days of storage | TSP2L | CNTRL2L | Difference | t | P value | Summary |
| Day 0 | 7.391 | 7.229 | -0.162 | 0.229 | P > 0.05 | ns |
| Day 3 | 8.533 | 8.883 | 0.349 | 0.495 | P > 0.05 | ns |
| Day 6 | 8.319 | 8.151 | -0.168 | 0.239 | P > 0.05 | ns |

## Appendix VI: Statistical Analyses for the Total Coliform Count (TCC) of Sabo Market

LA1L vs LA2L

| Days of storage | LA 1L | LA 2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 5.737 | 5.122 | -0.615 | 0.986 | P > 0.05 | ns |
| Day 3 | 8.686 | 8.488 | -0.198 | 0.317 | P > 0.05 | ns |
| Day 6 | 8.455 | 8.431 | -0.024 | 0.039 | P > 0.05 | ns |

LA1L vs TSP1L

| Days of storage | LA 1L | TSP 1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 5.737 | 6.735 | 0.998 | 1.601 | P > 0.05 | ns |
| Day 3 | 8.686 | 8.728 | 0.043 | 0.068 | P > 0.05 | ns |
| Day 6 | 8.455 | 8.246 | -0.209 | 0.335 | P > 0.05 | ns |

LA1L vs CNTRL1L

| Days of storage | LA 1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 5.737 | 6.750 | 1.013 | 1.625 | P > 0.05 | ns |
| Day 3 | 8.686 | 8.761 | 0.075 | 0.120 | P > 0.05 | ns |
| Day 6 | 8.455 | 8.326 | -0.129 | 0.207 | P > 0.05 | ns |

LA2L vs TSP2L

| Days of storage | LA2L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 5.122 | 6.701 | 1.579 | 2.533 | P < 0.05 | * |
| Day 3 | 8.488 | 8.729 | 0.242 | 0.386 | P > 0.05 | ns |
| Day 6 | 8.431 | 8.137 | -0.294 | 0.472 | P > 0.05 | ns |

LA2L vs CNTRL2L

| Days of storage | LA2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 5.122 | 7.502 | 2.380 | 3.817 | P<0.001 | *** |
| Day 3 | 8.488 | 8.603 | 0.115 | 0.184 | P > 0.05 | ns |
| Day 6 | 8.431 | 7.754 | -0.677 | 1.085 | P > 0.05 | ns |

TSP1L vs TSP2L

| Days of storage | TSP1L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.735 | 6.701 | -0.034 | 0.055 | P > 0.05 | ns |
| Day 3 | 8.728 | 8.729 | 0.001 | 0.001 | P > 0.05 | ns |
| Day 6 | 8.246 | 8.137 | -0.109 | 0.175 | P > 0.05 | ns |

TSP1L vs CNTRL 1L

| Days of storage | TSP1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.735 | 6.750 | 0.015 | 0.024 | P > 0.05 | ns |
| Day 3 | 8.728 | 8.761 | 0.033 | 0.052 | P > 0.05 | ns |
| Day 6 | 8.246 | 8.326 | 0.080 | 0.128 | P > 0.05 | ns |

TSP2L vs CNTRL 2L

| Days of storage | TSP2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.701 | 7.502 | 0.801 | 1.284 | P > 0.05 | ns |
| Day 3 | 8.729 | 8.603 | -0.126 | 0.202 | P > 0.05 | ns |
| Day 6 | 8.137 | 7.754 | -0.383 | 0.613 | P > 0.05 | ns |

## Appendix VII: Statistical Analyses for the Aerobic Plate Count (APC) of Samaru Market

LA1L vs LA2L

| Days of storage | LA 1L | LA 2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.699 | 8.041 | 0.342 | 0.563 | P > 0.05 | ns |
| Day 3 | 7.335 | 7.924 | 0.589 | 0.971 | P > 0.05 | ns |
| Day 6 | 8.638 | 8.118 | -0.519 | 0.856 | P > 0.05 | ns |

LA1L vs TSP1L

| Days of storage | LA 1L | TSP 1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.699 | 9.175 | 1.476 | 2.433 | P < 0.05 | * |
| Day 3 | 7.335 | 8.955 | 1.620 | 2.671 | P < 0.05 | * |
| Day 6 | 8.638 | 8.853 | 0.215 | 0.354 | P > 0.05 | ns |

LA1L vs CNTRL1L

| Days of storage | LA 1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.699 | 7.062 | -0.638 | 1.051 | P > 0.05 | ns |
| Day 3 | 7.335 | 8.513 | 1.178 | 1.941 | P > 0.05 | ns |
| Day 6 | 8.638 | 8.404 | -0.233 | 0.385 | P > 0.05 | ns |

LA2L vs TSP2L

| Days of storage | LA2L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.041 | 7.219 | -0.822 | 1.355 | P > 0.05 | ns |
| Day 3 | 7.924 | 9.148 | 1.223 | 2.017 | P > 0.05 | ns |
| Day 6 | 8.118 | 9.088 | 0.969 | 1.598 | P > 0.05 | ns |

LA2L vs CNTRL2L

| Days of storage | LA2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.041 | 7.448 | -0.593 | 0.977 | P > 0.05 | ns |
| Day 3 | 7.924 | 7.497 | -0.428 | 0.705 | P > 0.05 | ns |
| Day 6 | 8.118 | 8.540 | 0.422 | 0.695 | P > 0.05 | ns |

TSP1L vs TSP2L

| Days of storage | TSP1L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 9.175 | 7.219 | -1.956 | 3.224 | P< 0.01 | ** |
| Day 3 | 8.955 | 9.148 | 0.193 | 0.317 | P > 0.05 | ns |
| Day 6 | 8.853 | 9.088 | 0.235 | 0.387 | P > 0.05 | ns |

TSP1L vs CNTRL 1L

| Days of storage | TSP1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 9.175 | 7.062 | -2.113 | 3.484 | P< 0.01 | ** |
| Day 3 | 8.955 | 8.513 | -0.443 | 0.730 | P > 0.05 | ns |
| Day 6 | 8.853 | 8.404 | -0.448 | 0.739 | P > 0.05 | ns |

TSP2L vs CNTRL 2L

| Days of storage | TSP2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 6.701 | 7.448 | 0.229 | 0.378 | P > 0.05 | ns |
| Day 3 | 8.729 | 7.497 | -1.651 | 2.722 | P < 0.05 | * |
| Day 6 | 8.137 | 8.540 | -0.548 | 0.903 | P > 0.05 | ns |

## Appendix VIII: Statistical Analyses for the Aerobic Plate Count (APC) of Tudun-wada Market

LA1L vs LA2L

| Days of storage | LA 1L | LA 2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.386 | 8.478 | 1.092 | 2.424 | P < 0.05 | * |
| Day 3 | 8.782 | 8.698 | -0.084 | 0.187 | P > 0.05 | ns |
| Day 6 | 8.774 | 8.158 | -0.616 | 1.368 | P > 0.05 | ns |

LA1L vs TSP1L

| Days of storage | LA 1L | TSP 1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.386 | 8.292 | 0.906 | 2.012 | P > 0.05 | ns |
| Day 3 | 8.782 | 8.858 | 0.075 | 0.167 | P > 0.05 | ns |
| Day 6 | 8.774 | 8.895 | 0.121 | 0.268 | P > 0.05 | ns |

LA1L vs CNTRL1L

| Days of storage | LA 1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.386 | 8.567 | 1.181 | 2.622 | P < 0.05 | * |
| Day 3 | 8.782 | 8.939 | 0.157 | 0.348 | P > 0.05 | ns |
| Day 6 | 8.774 | 8.883 | 0.108 | 0.241 | P > 0.05 | ns |

LA2L vs TSP2L

| Days of storage | LA2L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.478 | 8.644 | 0.167 | 0.370 | P > 0.05 | ns |
| Day 3 | 8.698 | 8.963 | 0.264 | 0.587 | P > 0.05 | ns |
| Day 6 | 8.158 | 8.989 | 0.831 | 1.845 | P > 0.05 | ns |

LA2L vs CNTRL2L

| Days of storage | LA2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.478 | 9.088 | 0.610 | 1.355 | P > 0.05 | ns |
| Day 3 | 8.698 | 9.008 | 0.310 | 0.688 | P > 0.05 | ns |
| Day 6 | 8.158 | 8.914 | 0.756 | 1.678 | P > 0.05 | ns |

TSP1L vs TSP2L

| Days of storage | TSP1L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.292 | 8.644 | 0.353 | 0.783 | P > 0.05 | ns |
| Day 3 | 8.858 | 8.963 | 0.105 | 0.233 | P > 0.05 | ns |
| Day 6 | 8.895 | 8.989 | 0.094 | 0.209 | P > 0.05 | ns |

TSP1L vs CNTRL 1L

| Days of storage | TSP1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.292 | 8.567 | 0.275 | 0.611 | P > 0.05 | ns |
| Day 3 | 8.858 | 8.939 | 0.082 | 0.181 | P > 0.05 | ns |
| Day 6 | 8.895 | 8.883 | -0.013 | 0.028 | P > 0.05 | ns |

TSP2L vs CNTRL 2L

| Days of storage | TSP2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.644 | 9.088 | 0.443 | 0.985 | P > 0.05 | ns |
| Day 3 | 8.963 | 9.008 | 0.046 | 0.102 | P > 0.05 | ns |
| Day 6 | 8.989 | 8.914 | -0.075 | 0.167 | P > 0.05 | ns |

## Appendix IX: Statistical Analyses for the Aerobic Plate Count (APC) of Sabo Market.

LA1L vs LA2L

| Days of storage | LA 1L | LA 2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.023 | 7.966 | -0.057 | 0.138 | P > 0.05 | ns |
| Day 3 | 8.830 | 8.595 | -0.235 | 0.570 | P > 0.05 | ns |
| Day 6 | 8.775 | 8.838 | 0.063 | 0.152 | P > 0.05 | ns |

LA1L vs TSP1L

| Days of storage | LA 1L | TSP 1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.023 | 8.215 | 0.193 | 0.467 | P > 0.05 | ns |
| Day 3 | 8.830 | 8.879 | 0.049 | 0.119 | P > 0.05 | ns |
| Day 6 | 8.775 | 8.627 | -0.148 | 0.360 | P > 0.05 | ns |

LA1L vs CNTRL1L

| Days of storage | LA 1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.023 | 8.622 | 0.599 | 1.454 | P > 0.05 | ns |
| Day 3 | 8.830 | 8.845 | 0.015 | 0.036 | P > 0.05 | ns |
| Day 6 | 8.775 | 8.607 | -0.168 | 0.408 | P > 0.05 | ns |

LA2L vs TSP2L

| Days of storage | LA2L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.966 | 8.426 | 0.460 | 1.116 | P > 0.05 | ns |
| Day 3 | 8.595 | 8.813 | 0.218 | 0.530 | P > 0.05 | ns |
| Day 6 | 8.838 | 8.588 | -0.249 | 0.605 | P > 0.05 | ns |

LA2L vs CNTRL2L

| Days of storage | LA2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 7.966 | 8.550 | 0.584 | 1.417 | P > 0.05 | ns |
| Day 3 | 8.595 | 8.752 | 0.157 | 0.380 | P > 0.05 | ns |
| Day 6 | 8.838 | 8.556 | -0.282 | 0.683 | P > 0.05 | ns |

TSP1L vs TSP2L

| Days of storage | TSP1L | TSP2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.215 | 8.426 | 0.211 | 0.512 | P > 0.05 | ns |
| Day 3 | 8.879 | 8.813 | -0.066 | 0.160 | P > 0.05 | ns |
| Day 6 | 8.627 | 8.588 | -0.038 | 0.093 | P > 0.05 | ns |

TSP1L vs CNTRL 1L

| Days of storage | TSP1L | CNTRL1L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.215 | 8.622 | 0.407 | 0.987 | P > 0.05 | ns |
| Day 3 | 8.879 | 8.845 | -0.034 | 0.083 | P > 0.05 | ns |
| Day 6 | 8.627 | 8.607 | -0.020 | 0.049 | P > 0.05 | ns |

TSP2L vs CNTRL 2L

| Days of storage | TSP2L | CNTRL2L | Difference | t | P value | Summary |
|---|---|---|---|---|---|---|
| Day 0 | 8.426 | 8.550 | 0.124 | 0.301 | P > 0.05 | ns |
| Day 3 | 8.813 | 8.752 | -0.062 | 0.150 | P > 0.05 | ns |
| Day 6 | 8.588 | 8.556 | -0.033 | 0.079 | P > 0.05 | ns |

**Appendix X: Statistical Analysis to Compare the Microbial Load (TCC and APC) between the Three Different Markets**.

| Location | TCC | APC | Difference | P value | Summary |
|---|---|---|---|---|---|
| Samaru | 7.168 | 8.204 | -1.036 | 0.0002 | *** |
| Tudun-wada | 7.753 | 8.684 | -0.932 | 0.0006 | *** |
| Sabo | 7.773 | 8.586 | -0.813 | 0.0048 | ** |

***= Values are statistically significant (P<0.001); **= Values are statistically significant (P<0.01).

## Appendix XI: Media and Reagents

**Lactic acid** (a variable mixture of D- and L- lactic acid acids) 'Analar$^{R}$

$CH_3$-CHOH-COOH; M.W.: 90.08 Minimum assay (after hydrolysis of anhydrides)- 88.0%; 500 ml

Components:

Sulphated ash - 0.01%

Volatile fatty acid passes test

Chloride (Cl) - 0.01%

Sulphate ($SO_4$) - 0.005%

Arsenic (AS) - 0.00001%

Calcium (Ca) - 0.02%

Copper (Cu) - 0.0001%

Iron (Fe) - 0.0001%

Lead (Pb) - 0.0001%

Zinc (Zn) - 0.001%

**Trisodium Phosphate (TSP)**, (Sigma- Aldrich Chemicals, Germany)

$Na_3PO4.\ 12H_20$

Assay 98.0 – 102.0%

Excess Alkali ≤ 2.5%

Heavy metals ≤ 0.001%

Insolubles ≤ 0.01%

Arsenic (AS) ≤ 5ppm

Chlorine ≤ 0.001%

Iron ≤ 0.001%

Sulphides ≤ 0.01%

**Buffered Peptone Water (BPW)** (Oxoid, Basingtoke, England)

pH 7.2 ± 0.2 at 25 $^{O}$C, 500 ml

Formular (g/l)

Peptone – 10.0

Sodium Chloride – 5.0

Di- sodium phosphate – 3.5

Potassium dihydrogen phosphate – 1.5

**Eosin Methylene Blue (EMB) (**Oxoid, Basingtoke, England**)**

pH 6.8 ± 0.2 at 25$^{O}$C, 500ml

Formular (g/l)

Peptone – 10.0

Lactose – 10.0

Di- potassium hydrogen phosphate – 2.0

Eosin Y – 0.4

Meythylene blue – 0.06

Agar – 15.0

**Sodium Chloride (NaCl)**

Minimum assay (ex Cl) after ignition – 99.5%

Loss on drying ay 105 $^{O}$C- 1.0%

Sulphate ($SO_4$) – 0.02%

Ammonia ($NH_3$) – 0.002%

Iron (Fe) – 0.002%

Lead (Pb) – 0.005%

Potassium (K) – 0.02%

**MacConkey Agar** (Oxoid, Basingtoke, England)

pH 7.4± 0.2 at 25$^{O}$C

Formular (g/l)

Peptone – 20.0

Lactose – 10.0

Bile salts – 5.0

Sodium Chloride – 5.0

Neutral Red – 0.075

Agar – 12.0

**Nutrient Agar** (Oxoid, Basingtoke, England)

pH 7.0 ± 0.2 at 25 $^{O}$C

Formular (g/l)

'Lab – lemco' powder – 1.0

Yeast extract – 2.0

Peptone – 5.0

Sodium Chloride – 5.0

Agar – 15.0

**Simmons Citrate Agar** (Oxoid, Basingtoke, England)

pH 7.0 ± 0.2 at 25 $^{O}$C

Formular (g/l)

Magnesium Sulphate - 0.2

Ammonium dihydrogen Phosphate – 0.8

Sodium Amonium Phoshate – 0.8

Sodium Citrate, tribasic – 2.0

Sodium Chloride – 5.0

Bromothymol blue – 0.08

Agar – 15.0

**Methyl Red Voges Proskuer Medium** (M.R.V.P) (Oxoid, Basingtoke, England)

pH 6.9 ± 0.2 at 25 $^{O}$C

Formular (g/l)

Peptone – 7.0

Glucose – 5.0

Phosphate buffer – 5.0

**S.I.M** (Oxoid, Basingtoke, England)

pH 7.3± 0.2 at 25 $^{O}$C

Formular (g/l)

Tryptone – 20.0

Peptone – 6.1

Ferrous Ammonium Sulphate – 0.2

Sodium thiosulphate – 0.2

Agar – 3.5

**Urea Agar Base** (Oxoid, Basingtoke, England)

pH 6.8 ± 0.2 at 25 $^{O}$C

Formular (g/l)

Peptone – 1.0

Glucose – 1.0

Sodium Chloride – 5.0

Di –Sodium Phosphate – 1.2

Potassium dihydrogen phosphate – 0.8

Phenol Red – 0.012

Agar – 15.0

**Triple Sugar Iron Agar** ( Oxoid, Basingtoke, England)

pH 7.4 ± 0.2 at 25 $^{O}$C

Formular (g/l)

'Lab – lemco' powder – 3.0

Yeast extract – 3.0

Peptone – 20.0

Sodium Chloride – 5.0

Lactose – 10.0

Sucrose 10 .0

Glucose – 1.0

Ferric Citrate – 0.3

Sodium thiosulphate – 0.3

Phenol Red – 0.024

Agar – 12.0