To investigate the potential of *Moringa oleifera* leaf extract and broccoli (*Brassica oleracea*) leaf extract as Sodium sulphite replacers in preserving minced meat

MICHELLE YEMURAI NYATI

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BY

MICHELLE YEMURAI NYATI

(R135988Z)

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ABSTRACT

Minced meat is a nutritionally dense and highly perishable food, therefore there is need for preserving it. Most artificial preservatives currently used to prevent food spoilage have been reported to cause various health problems hence the need to use natural preservatives. *Moringa oleifera* and *Brassica oleracea* (broccoli) leaf extracts have proved their potential to be used as natural preservatives. The main objective of this research was to determine the antimicrobial efficacy of *Moringa oleifera* and broccoli leaf extracts as minced meat preservatives. Seven minced meat samples were prepared with different preservative concentrations. The first one had no preservative, the second one was preserved with 0.1% sodium sulphite, the third one was preserved with 1% *Moringa oleifera* leaf extract, the fourth one was preserved with 2% *Moringa oleifera* leaf extract, the fifth one was preserved with 1% broccoli leaf extract, the sixth one was preserved with 2% broccoli leaf extract and the seventh one was preserved with 1% *Moringa oleifera* and 1% broccoli leaf extracts. The minced meat samples were tested periodically (after 1, 12, 24, 48 and 72 hours) for microbial load (Total Bacterial Count, Coliforms, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*), colour stability and sensory analysis. *Escherichia coli* and *Salmonella* were not detected in all samples. The Total Bacteria, *Staphylococcus aureus* and Coliform counts for the samples varied from 4.3- 5.98 log CFU/gram, 1.32- 3.91 log CFU/gram and 3.4- 5.3 log CFU/gram respectively. The colour a*, L* and b*- values had ranges between 5-14, 53.2- 44.2 and 12- 15 respectively. The differences were compared using Graph pad prism 4 one way ANOVA for significant difference (α 0.05). It was concluded that there was no significant difference in the shelf life of the minced meat preserved with broccoli and *Moringa oleifera* leaf extracts to the one preserved with sodium sulphite.

DECLARATION

I, **Michelle Y Nyati** hereby declare that I am the sole author of this dissertation. I authorize Midlands State University to lend this dissertation to other institutions or individuals for the purpose of scholarly research.

Signature……Date…….

APPROVAL

This dissertation entitled "**To investigate the potential of *Moringa oleifera* leaf extract and broccoli (*Brassica oleracea*) leaf extract as Sodium sulphite replacers in preserving minced mea**t" by **Michelle Y Nyati** meets the regulations governing the award of the Honors Degree of Food Science and Nutrition of the Midlands State University, and is approved for its contribution to knowledge and literal presentation.

Supervisor ……

Date ……

DEDICATION

This work is dedicated to my parents.

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF STUDY

Meat is generally considered a nutritionally dense food which has a high quality proteins, important minerals and vitamins (Rao, Thulasi and Ruban, 2009). However it is a highly perishable product which under goes spoilage from time of slaughter till consumption, therefore meat preservation is important in delaying spoilage, extend life of the product and improve product quality. Different preservation techniques are used such as drying, smoking, brining and canning meat. Recently these techniques are being replaced by new methods such as chemical, bio-preservative and non-thermal techniques (Aymerich, Picouet, &Monfort, 2008). Use of synthetic food preservatives is common in the food industry. However, food additives such as monosodium glutamate, aspartame, saccharin, sodium cyclamate, sulfites, nitrates, nitrites and antibiotics have been associated with negative health effects such as headache, nausea, weakness, mental retardation, seizures, cancer and anorexia (Rangan and Barceloux, 2009).One of the most common and widely used chemical preservatives in the meat industry is sodium sulphite, which is a product of SO_2 scrubbing, a part of the flue gas desulphurization process. Sodium sulphite is used as a food preservative to preserve minced meat, it preserves the meat colour and has antimicrobial effects on the minced meat. Whilst it acts as a preservative, it also has health implications for about 10% of all consumers (Msagati, 2012).

More recently, of interest is the use and application of natural organic by-products and plant extracts as preservatives and as antioxidants in foods. Plants like *Moringa oleifera* have been reported to exhibit inhibitory effect on many food borne pathogens. *Moringa oleifera* belongs to a monogenetic family, the Moringaceae (Sunil, 2006). *Moringa oleifera* is also known as "Miracle Tree", it has leaves which are reported to possess various biological activities, including hypocholesterolemic, antidiabetic, hypertensive agent and regulate thyroid hormone (Tahiliani and Kar, 2004). A large number of researches on the nutritional qualities of *Moringa oleifera* leaf report that it is rich in β-caroteine, calcium, iron, vitamin C and potassium(Fahey, 2005).Further study showed that *Moringa oleifera* leaves possess inhibitory properties and thus can serve as an alternative preservative in foods (Anthonia, 2012).The phenolic and flavonoid

properties of *Moringa oleifera* extract can hinder the growth of pathogenic microorganisms and may extend the shelf life of food (Hemen, Johnson, Ujah and Udenze, 2013).

Another vegetable group of interest is *Brassicaceae* vegetables, they have been reported to have anticancer and antioxidant properties (Keck and Finley, 2004). *Brassica oleracea* L. var. *italica* (broccoli) belongs to the *Brassicaceae* family. The health benefits of broccoli are derived from a unique mixture of organic compounds, nutrient, vitamins and minerals that are found in broccoli. In terms of unique organic compounds broccoli is a rich source of phytonutrients, glucosinolates and flavonoids (Mahro and Timm, 2007). Broccoli has anticancer properties due to presence of 3,3-diindolylmethane, a potent modulator of the innate immune response system (Kim et al., 2013) .It also has active compounds like isothiocyanates therefore making it a potential antimicrobial. Dimayuga and Garcia (1991), investigated antibacterial activity against food borne pathogen using extract of petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous of broccoli and determined the Minimum inhibitory concentration (MIC) values to be approximately 10 - 320 $\mu g\ ml^{-1}$.

1.2 PROBLEM STATEMENT

The use of sodium sulphate as a minced meat preservative may lead to health hazards. Sodium sulphite has been associated with symptoms such as mild headaches or as severe as anaphylactic shock and they can occur within 15 to 20 minutes after ingestion (Roller et al., 2002). Most sulphites reactions occur in people with medical conditions such as asthma, liver and kidney dysfunction (Seetaramaiah et al., 2011). Sulphites preserve food by preventing bacterial growth; however they have a negative effect on the nutritional quality of food as they have been proven to destroy vitamin B1, thiamine, which is present in large amounts in meat, dairy and cereal products (Saulo, 1994).

Given the challenges associated with the consumption of food with sodium sulphite as a preservative, antimicrobial extracts from plants or vegetables can offer natural sources of preservatives with potential application in the food industry. Broccoli extract has been reported to be a rich source of various phenolic compounds like polyphenols (Kim et al., 2013). The extracts can be incorporated into meat products, and these can prolong quality and colour stability of minced meat. According to the research by Singh and Bhat (2003) *Moringa oleifera* leaf extract proved its potential to be used as a natural preservative in different foods

(Chidzonga, 2015). It showed a broad-spectrum antibacterial activity with a zone of inhibition of 0 to 22 mm and antifungal activity with a zone of inhibition of 8 to 14 mm. The researcher will focus on the use of *Moringa oleifera* and Broccoli leaf extracts as potential natural antimicrobial compounds for use in minced meat preservation. Their ability to control food borne pathogens and maintain the colour of minced meat will be investigated.

1.3 SIGNIFICANCE OF STUDY

To the meat industry

This study will provide the meat industry with healthier and safer alternatives for meat preservation increasing the healthy consumer base and serving.

To the consumer

The research will potentially improve consumer health and customer satisfaction as they request the use of natural preservatives to replace chemical compounds.

The researcher

The study will widen the researcher's knowledge on microbiological aspects of food preservation as well as increase the authors' knowledge on application of natural preservatives.

1.4 OBJECTIVES

1.4.1 Main Objective

- To determine the antimicrobial efficacy of *Moringa oleifera* and broccolileaf extracts as minced meat preservatives.

1.4.2 Specific objectives

- To determine Total Bacterial Count, coliforms, *Salmonella and Escherichia coli* in minc ed meat prepared with different preservatives concentrations of *Moringa oleifera* and bro ccoli leaf extracts.
- To determine the difference in colour changes of minced meat preserved with *Moringa o leifera* and broccoli leaf extracts compared to Sodium sulphite.
- To determine the microbial shelf life of minced meat preserved with *Moringa oleifera* an d broccoli leaf extracts.
- To evaluate the sensory attributes of minced meat preserved with *Moringa oleifera* and b roccoli leaf extracts as compared to sodium sulphite.

1.5 HYPOTHESIS

H_O1: There is no significant difference in the bacterial load of minced meat preserved with *Moringa oleifera* leaf extract and the one preserved with sodium sulphite.

H_O2: There is no significant difference in the quality and shelf life of minced meat preserved with *Moringa oleifera* and broccoli leaf extracts to the one preserved with sodium sulphite.

H_O3: There is no significant difference in the bacterial load of minced meat preserved with broccoli leaf extract and the one preserved with sodium sulphite.

1.6 DELIMITATIONS

Experiments will be carried out at Midlands State University Laboratory, from 7 March to 21 March 2017.

CHAPTER TWO: LITERATURE REVIEW

2.1 GROUND BEEF

Ground beef is basically beef that has been ground or finely chopped by a meat grinder or a chopping knife. The grinding process tenderizes cuts of beef which are very tough, and grinding fatty cuts in with lean cuts helps to reduce the dryness and improve the flavour of traditionally dry cuts. Ground beef is more perishable than whole muscle cuts of meat and should be handled with particular care (Rao, Thulasi and Ruban, 2009). In addition, if any bacteria are present on the surface, the grinding process mixes it throughout the product therefore ground meat has food safety concerns not associated with whole cuts of meat. In a whole cut from an animal, the interior of the meat is essentially sterile even before cooking thus any bacterial contamination is on the outer surface of the meat (Magnus, 1981). If ground beef is undercooked, there is a significant chance that enough pathogenic bacteria will survive to cause illness, moreover the warming will speed the reproduction of bacteria hence it is essential for ground meat to be preserved appropriately (Kim et al., 2013).

2.1.1 Shelf life of ground beef

Shelf life is defined as the period of time between packaging of a product and its end use in which product properties remain acceptable to the product user. Shelf-life properties may include appearance, texture, flavour, colour and nutritive value (Singh and Singh, 2005).When considering the shelf-life of a meat product like ground beef; some people make a distinction between case-life and shelf-life. Case-life (also referred to as colour shelf-life or display-life) is described as the length of time meat can be displayed under refrigeration before a colour change occurs. This colour change from the bright cherry-red colour of beef to another colour such as brown is caused by a change in the protein myoglobin due to oxidation (Brooks, 2007). While this colour change is not harmful and does not denote spoilage, it results in a colour customers find undesirable. Shelf-life of beef is often used to describe the length of time before the product will spoil, or more specifically the time required for spoilage organisms to reach an unacceptable level. The meat industry works diligently to prevent, reduce and eliminate both pathogenic and spoilage bacteria before meat is delivered to consumers. Fresh ground beef can be stored for up to 2 days in a refrigerator at 4^{o}C (Toldra, 2010).

2.2 SPOILAGE OF MINCED MEAT

Meat is a nutritious, protein-rich food which is highly perishable and has a short shelf-life unless proper preservation methods are used. It is the first choice source of animal protein for many people all over the world. Consumption of meat is continuously increasing worldwide (Heinz and Hautzinger, 2007). Spoilage of minced meat can be considered as an ecological phenomenon that encompasses changes of the available components during proliferation of bacterial present in the microbial association of the stored meat (Klein and DeWaal, 2013).

The main causes of minced meat spoilage after slaughtering and during processing and storage are microorganisms, lipid oxidation and autolytic enzymatic spoilage. Minced meat preservation becomes necessary for transporting meat without spoiling of texture, colour and nutritional value. Minced meat spoilage can be considered as any change which renders minced meat unacceptable for human consumption (Klein and DeWaal, 2013). Microbial growth causes colour defects, changes in texture, development of off-flavour, off-odour and slime making the meat unacceptable.

2.3 PATHOGENIC BACTERIA IN MEAT

Pathogenic bacteria are bacteria which are capable of causing disease. Humans are generally most interested in the species of bacteria which can cause disease in humans, although these bacteria can also infect other animals and plants. Some notable pathogenic bacteria include *Streptococcus, Staphylococcus, Salmonella,* and *Escherichia coli*, among many others. The majority of food-borne illnesses results from microbiological hazards caused by pathogenic microorganisms (Barbara and Grahame, 2000).

2.3.1Escherichia coli

E. coli is a gram-negative, non-spore forming short rod-shaped bacterium capable of growth and gas production at 45.5^{o}C. *E. coli* strains are harmless inhabitants of the gastrointestinal tract of man and animals. However, several food borne pathogenic strains of *E. coli* are known to exist (Kornacki and Marth, 1982).*E. coli* O157:H7 and other enterohemorrhagic *E. coli*

produce a toxin(s) after it implants in the colon and colonizes it resulting in illness. Pre formed toxins have not been shown to occur in foods or cause human disease, hence this organism is considered to be "toxico-infectious" agent , as opposed to an invasive pathogen (such as *Salmonella* spp.). However, some evidence for an invasive mechanism has been reported (Doyle, Beuchat and Montville, 1997). It is a difficult organism to manage from a public health standpoint, because of its low infectious dose which may be in part related to its substantial acid tolerance and its ability to survive low pH sometimes found in the stomach (Kornacki and Johnson, 2001). When beef is being slaughtered and processed, *E.coli* bacteria in the cows' intestines can get on meat, and this is how ground beef can be contaminated with *E.coli*.

2.3.2Salmonella

Salmonella species are gram-negative, rod-shaped, usually motile, members of the taxonomic family, Enterobacteriaceae. Despite great advances in molecular genetic approaches to identification and characterization these organisms are still serologically defined, that is by their somatic (O) and usually flagellar (H) and sometimes capsular (Vi) antigens (Holt and Chaubal, 2007). Over approximately 2,400 different serotypes are known to exist. The nomenclature of this microbe has gone through a number of changes resulting in some confusion. The pathogenic *Salmonella* is a life-threatening bacterium, and it is a leading cause of food-borne bacterial illnesses in humans. After Campylobacter, Salmonella is the second most predominant bacterial cause of food borne gastroenteritis worldwide (Kim and Foegeding, 1993). *Salmonella* can cause a number of disease syndromes including typhoid fever from *Salmonella typhi*. Other strains of *Salmonella* cause gastroenteritis, bacteraemia, and enteric or paratyphoid fever (Hensel, 2004). *Salmonella* is a facultative aerobe which means it can grow in oxygen rich environments. When you have meat exposed to *Salmonella*, it is able to grow. This exposure occurs due to contact with faeces, followed by meat handling.

2.3.4 Staphylococcus aureus

Staphylococci belong to the family Micrococcaceae. They are gram-positive spherical bacteria about 1μm in diameter that appear as grape-like clusters under the microscope. The grape-like configuration of staphylococci helps to distinguish staphylococci from streptococci that usually form chains because they divide in one plane only (Jablonski and Bohach, 2001). *S. aureus* is a common bacterial pathogen causing staphylococcal food poisoning (SFP), a leading cause of

food borne intoxication worldwide and accounts for an estimated 14% of all food borne illnesses in the United States (IAFP, 2009) . SFP is not attributed to ingestion of live bacterial cells but rather acquired from ingesting one or more heat stable pre formed staphylococcal enterotoxins (SEs) in foods contaminated with enterotoxin producing strains of staphylococci, principally, *S. aureus*. This type of food poisoning is classified as an intoxication since it does not require growth of the bacterium in the host. Indeed, numerous outbreaks have been caused by foods in which the organism has been killed but the heat-stable toxin remained. SEs are unique because they are not destroyed by heating including canning (Boor, 2001).*S. aureus* has a high salt tolerance, the bacteria lives in the nose, mouth and throat of humans as well as on the skin. Contamination of ground beef with *S. aureus* results from the failure to store the ground beef properly.

2.3.5 Coliforms

Coliform bacteria are microscopic organisms that originate in the intestinal tract of warm blooded animals and are also present in soil and vegetation. Total coliform bacteria are generally harmless however; their presence in meat indicates the possibility that disease causing bacteria, viruses or parasites (pathogens) are also present in the meat. Coliform bacteria are relatively simple to identify, are present in much larger numbers than more dangerous pathogens, and react to the natural environment and treatment processes similarly to pathogens. By observing coliform bacteria, the increase or decrease of many pathogenic bacteria can be estimated (Ray, 2004). Unhygienic practices during the production and handling of ground beef results in the contamination of the ground beef with coliforms.

2.4 FOOD PRESERVATION

Before the advent of preservatives, food was placed in containers such as clay jars to keep them from spoiling. Methods of food preservation have been an important part of food technology and they are designed to prevent chemical and quality changes caused by the natural spoilage flora which is present on any food. Traditional procedures for preserving food include drying, salting, smoking, pickling and a combination of these procedures. Public acceptance of salting and pickling dates back to the Babylonians some 3000 years B.C. Technologies introduced in the 19th century for preservation were heat sterilisation and meat dehydration. Deep freezing, cold-air cooling and cold pickling came to the fore at the beginning of the 20th century and

were followed by the use of irradiation, chemical preservatives and the disinfection of storage and manufacturing materials (Nychas, Skandamis, Tassou and Koutsoumanis, 2008).

2.4.1 Meat preservation

Fresh meat is considered to be one of the most perishable foods. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens. It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality (Aymerich, Picouet and Monfort, 2008). Preservation methods involve application of measures to delay or prevent certain changes which make meat unusable as a food or which downgrade some quality aspect of it. The pathways by which such deterioration can occur are diverse and these include microbial, physical and chemical processes (Mor-Mur and Yuste, 2003). With the increased demand for high quality, safety, convenience, fresh appearance and an extended shelf life in fresh meat products, alternative non-thermal preservation technologies such as super chilling, high hydrostatic pressure, active packaging and natural bio-preservatives have been proposed and investigated.

Meat preservation became necessary for transporting meat for long distances without spoiling of texture, colour and nutritional value after the development and rapid growth of super markets (Nychas et al., 2008). The aims of preservation methods are to inhibit the microbial spoilage and to minimize the oxidation and enzymatic spoilage. Traditional methods of meat preservation such as drying, smoking, brining and canning have been replaced by new preservation techniques such as chemical, bio-preservative and non-thermal techniques. Meat preservation methods can be categorized into three methods; these are controlling water activity, controlling temperature and the use of chemical or bio preservatives (Zhou, Xu and Liu, 2010). A combination of these preservation techniques can be used to diminish the process of spoilage (Bagamboula, Uyttendaele and Debevere, 2004).

Principles of preservation

Principles of preservation include prevention or delay of microbial decomposition which is achieved by keeping out microorganisms which is asepsis, removal of microorganisms through filtration, hindering the growth and activity of microorganisms using low temperature or drying

and killing the microorganisms using heat or radiation. Other principles of preservation include prevention or delay of self-decomposition of food which is achieved by the destruction and inactivation of food enzymes, as well as prevention of damage caused by insects, animals and mechanical causes (Nychas et al., 2008).

2.4.2 Types of Preservation Techniques in meat

They are six main types of preservation techniques in meat and these include cooking, dehydration, freezing, chemical, irradiation and fermentation (Brody, 2009).

1. Freezing

Optimum temperature for freezing is zero degrees Celsius or less. When frozen, the enzyme activity of the microorganism is inactivated and this results in inhibition of the meat spoilage. Frozen meat can even last up to 12 months depending with the type of meat. Thawing of the frozen meat prior to cooking should not be done at room temperature as it will lead to meat spoilage, rather it should be placed in a refrigerator for about one hour.

2. Cooking

There are two types of cooking methods used preserve meat and these are sterilized cooking and pasteurised cooking. During pasteurised cooking, meat is cooked at 65.5 – 70.6°C, this kills most (not all) of the microorganisms present in the meat. After pasteurized cooking the product can be served or if it is to be served later on it must be refrigerated, just before serving it has to be heated. Sterilised cooking involves cooking meat at 121°C under pressure, this kills all the micro-organisms present in the meat. Canned products are normally cooked in this method.

3. Chemical treatment

Chemical inhibit microbial growth in meat. Some of the chemicals used for such purpose are common salt, Sodium Nitrite, Sodium sulphite and Sodium Lactate. Advantages of adding a chemical is that it increases shelf life of product, develop flavour, and impart pink cured meat colour.

4. Fermentation

In fermentation the sugar present in the meat is converted into acid, microbes involved are lactic acid bacteria, which produces lactic acid, which reduces the pH in the meat and microbial

growth get inhibited preventing the meat spoilage. Fermentation adds tangy flavour and there is special texture development.

5. Irradiation

Is a new process to make food safer, it works by exposing the food to radiant energy and destroys most (not all) microbes. Advantages of this are food remain nutritious, reduces spoilage, and irradiated meat is safe to eat but prior to consumption the meat will have to be cooked.

2.5 PRESERVATIVES

Preservatives are in the category of food additives. The other food additive categories include nutritional additives, texturing agents, flavouring agents, miscellaneous additives and colouring agents (Belcher, 2006). A definition for additives, according to the Food Protection Committee of the Food and Nutrition Board (U.S.) is: "a substance or mixture of substances, other than basic foodstuff, which is present in a food as a result of any aspect of production, processing, storage, or packaging. The term does not include chance contaminants" (Belcher, 2006). The use of preservatives is to retard both biological and chemical deterioration of foods. Preservatives used to prevent biological deterioration are the antimicrobials, and those used to prevent chemical deterioration include anti browning compounds, antioxidants, and anti-staling compounds, (Coma, 2008).

Preservatives prolong the shelf-life of food, cosmetics and pharmaceuticals by preventing their spoilage. Antimicrobials such as nitrites, nitrates, benzoates and sulfur dioxide destroy or delay the growth of bacteria, yeast and moulds. Antioxidants such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), and propyl gallate slow or stop the breakdown of fats and oils. Anti-enzymatic preservatives such as citric and erythorbic acids block the enzymatic processes such as ripening occurring in foodstuffs even after harvest (Massey, 1995). Natural substances like salt, sugar, vinegar and spices have been traditionally been used as preservatives. The majority of preservatives used today are artificial rather than natural. Several of them are toxic and several others have potential life-threatening side effects. Researchers have reported that artificial preservatives such as nitrates, benzoates, sulphites, sorbates, parabens, formaldehyde, BHT, BHA and several others can cause serious health hazards such as hypersensitivity, allergy, asthma, hyperactivity, neurological damage and cancer. Research has proven that several natural preservatives obtained from plants, animals,

microbes and minerals contain antioxidant, antimicrobial and anti-enzymatic properties (Barbut, 2002).

2.5.1 Classification of Preservatives

Preservatives are classified into two classes which are natural and chemical preservatives. Natural food preservatives are good to our health, and do not harm our health. They include salts, sugar, rosemary extracts, *Moringa oleifera* extracts and vinegar. Microbial preservatives are the preservatives which inhibit the growth of bacteria and fungi, or anti-oxidants such as oxygen absorbers, which inhibit the oxidation of food constituents.

Artificial preservatives are the chemical substances that stop the growth and activities of the microorganisms and help to preserve the foods for a longer time without affecting its natural characteristics. They include nitrites, benzoates, sulphites, sorbates and nitrates of sodium or potassium, glutamates and glycerides. The food standards regulations require that not more than one chemical preservative should be used in one particular food item. People consuming or using items containing more than one preservative are at risk of exposure to multiple chemicals (Hugas, Garriga, and Monfort, 2002). Natural and synthetic preservatives are further categorized into 3 types which are antimicrobials, antioxidants and anti-enzymatic preservatives.

Antimicrobials are some of the most important food preservatives. These destroy or delay the growth of bacteria, yeast and mould for example nitrites and nitrates prevent botulism in meat products. According to Diez, Santos, Jaime, and Rovira (2009) current research is on synthetic, natural occurring, and biologically derived antimicrobials. Research is, however, especially needed on the application of naturally occurring and biologically derived antimicrobials in food systems, this is because consumers are rejecting the use of chemical preservatives but still demand foods with an acceptable shelf-life (Massey, 1995). Anti-oxidants slow or stop the breakdown of fats and oils in food that occurs in the presence of oxygen leading to rancidity. Anti-enzymatic preservatives block the enzymatic processes such as ripening occurring in foodstuffs even after harvest, an example is rythorbic acid and citric acid stop the action of enzyme phenolase that leads to a brown colour on the exposed surface of cut fruits or potato (Barbut, 2002).

2.6 SODIUM SULPHITE

Anand and Sati (2013) states that sulphites are food additives that help preserve freshness. Sodium sulphite in particular is commonly added to fresh produce and meats to help retain colour and preserve the meat. Sodium sulphite (sodium sulphite) is a soluble sodium salt of sulfurous acid (sulphite)with the chemical formulaNa_2SO_3. It is a product of sulfur dioxide scrubbing, a part of the flue-gas desulfurization process. Sodium sulphite is made industrially by reacting sulfur dioxide with a solution of sodium carbonate, and it occurs as two forms that is the crystal form (heptahyrate) called Sodium Sulphite (crystal) and the anhydrous form called Sodium Sulphite (anhydrous) (Furrer , Mayer and Gurny, 2002). Sodium Sulphite, when calculated on the anhydrous basis, contains not less than 95.0% of sodium sulphite (Na2SO3). It occurs as colourless to white crystals or as a white powder. Sodium sulphite can be identified by doing qualitative tests for sodium salt and for sulphite. Sulphites are used as preservatives in preserving minced meat and can also be used in dried fruits, biscuit dough, fruit juices and syrups, beer, fruit-based dairy desserts, cider and wine (Furrer, Mayer and Gurny, 2002). Sodium sulphite inhibits the growth and survival of undesirable microorganisms creating an inhospitable environment for pathogens. This can be achieved by the ability of the sodium to associate with water molecules, therefore reducing the water activity in the ground beef (Anand and Sati, 2013).

2.6.1 Toxicology of sodium sulphite

Msagati (2012) states that sulphite sensitivities can manifest in symptoms as mild as a headache or as severe as anaphylactic shock, and they can occur within 15 to 30 minutes after ingestion. Most reactions are mild, resulting in wheezing or respiratory irritation, but severe symptoms can include a narrowing of the airways and difficulty breathing, and emergency treatment may be required. Most reactions are of a respiratory nature, but symptoms of nausea, diarrhoea and abdominal pain have also been reported (Anand and Sati, 2013). A 1985 paper in the "Canadian Medical Association Journal" also reports that deaths in both Canada and the United States have been linked with sulphite exposure, although the mechanism by which they occurred is unclear. Sulphite containing food preservatives may cause severe allergic reactions and exacerbation of asthma. Sulphites have been reported to possibly cause neurological damage in rats and are potent irritants and allergens. The use of these toxic chemicals by pregnant women may adversely affect foetal brain development. Research has shown that the food additives used in hundreds of children's foods and drinks can cause temper tantrums and disruptive behaviour (Roller et al., 2002).

Currently, sulphites are not permitted in Canada as meat additives (DJC, 2009). Sulphur dioxide and the salts potassium bisulphite, potassium metabisulphite, sodium bisulphite, sodium metabisulphite and sodium sulphite collectively known as sulphites are removed from GRAS listing and they are not allowed for use as preservative in meat in the U.S. because the degradation of vitamin thiamine by sulphites (Saulo, 1994). Sulphites have also been linked with pruritus, urticaria and angioedema (Furrer, Mayer and Gurny, 2002). When fed to animals, sulphites have also been found to have a mutagenic action (Saulo, 1994).

2.7 NATURAL PRESERVATIVES

Natural preservatives offer greater advantages over artificial preservatives because they are non-toxic and have a wide range of health benefits. Extracts of basil, broccoli, neem, citrus, *Moringa oleifera* and rosemary are better alternatives to preservatives such as benzoic acid, sulphites, nitrates, MSG, BHA and BHT (Huang, Ou and Prior, 2005).

2.7.1 Broccoli

Broccoli (*Brassica oleracea*) is an edible green plant, which is also a cool season crop that performs poorly in hot weather. As a member of the crucifer family, broccoli is closely related to other cole crops, such as cabbage, cauliflower, and Brussels sprouts. The word broccoli comes from the Italian plural of broccolo, which means the flowering crest of a cabbage, and is the diminutive form of brocco, meaning "small nail" or "sprout" (Moreno et al., 2006). Broccoli is often boiled or steamed but may be eaten raw. Broccoli is classified in the Italica cultivar group of the species Brassica oleracea, it has large flower heads, usually green in colour, arranged in a tree-like structure branching out from a thick, edible stalk.

The mass of flower heads is surrounded by leaves. Broccoli resembles cauliflower, which is a different cultivar group of the same species. There are three commonly grown types of broccoli which are calabrese broccoli, sprouting broccoli and purple cauliflower. Calabrese broccoli has large (10 to 20 cm) green heads and thick stalks, sprouting broccoli has a larger number of heads with many thin stalks (16-18). Purple cauliflower is a type of broccoli which has a head shaped like cauliflower, but consisting of tiny flower buds which are sometimes purple (Faller and Fialho, 2009).

In the context of health-promoting foods, broccoli is also considered a source of bioactive phytochemicals (Moreno et al., 2006). In fact, the data available reveal broccoli to be a healthy food due to the beneficial biological effects of its phytochemicals (Boivin et al., 2009).

The Brassicaceae family vegetables are rich in glucosinolates. Glucosinolates, (alkyl-N-hydroximine sulphate esters with a β-D thioglucopyranosid group attached to the hydroximine carbon in Z-configuration relative to the sulphate group) have been reported to have detrimental activity against various types of cancers such as breast, colon and lung (Podsedek, 2007). These are also reported to have antibacterial and fungistatic activity. Over 120 different glucosinolates have been identified to this date. Glucosinolates may breakdown by the action of the endogenous enzyme myrosinase (thioglucoside glucohydrolase) to form isothiocyanates, nitriles thiocyanates, indoles and oxazolidinethiones (Valko, Rhodes and Moncol, 2006). Isothiocyanates and indoles in particular have been implicated to have anti-carcinogenic properties. There are clear indications that they block tumour initiation by modulating the activities of Phase I and Phase II biotransformation enzymes and increase the antioxidant effect and suppress tumors by forcing tumor cells to go for apoptosis (Lin and Chang, 2005).

2.7.1.2Antioxidant effect of broccoli

As it was pointed out by Huang et al., (2005) a general definition of antioxidant is rather straightforward as a sub-stance that opposes oxidation or inhibits reactions promoted by oxygen or peroxides, many of these substances being used as preservatives in various products (Podsedek, 2007). Broccoli is renowned for its vast range of non-enzymatic bioactive compounds, being rich in both nutritional antioxidants; vitamins C and E, and non-nutritional antioxidants; carotenoids, and phenolic compounds, particularly flavanoids (Lin and Chang, 2005). Broccoli is also rich in polyphenols, a large group of phytochemicals that are often considered the most abundant antioxidants in the diet (Faller and Fialho, 2009). Polyphenols cause interference with oxidation of lipids and other molecules by the rapid donation of hydrogen atoms to free radicals. The intermediates of the phenoxy radical are fairly stable and so prevent the initiation of further radical reactions. Valko, Rhodes and Moncol (2006) states that, flavanoids and their derivatives are the largest and most prominent group of polyphenols and are ideal scavengers of peroxyl radicals due to their specific reduction actions relative to alkyl peroxyl radicals, making them effective inhibitors of lipoperoxida-tion (Valko, Rhodes and Moncol, 2006). Broccoli has been reported to contain both flavonol and hydroxycinnamoyl derivatives (Vasanthi, Mukherjee and Das, 2009). Few studies have investigated anthocyanins

in broccoli which are the most prominent group of plant pigments among the coloured flavonoids and possess high antioxidant activity (AA) (Monero, Perez-Balibrea and Ferreres, 2010). Monero et al. (2010) studied the properties of acylated anthocyanins in broccoli and found the colour of purple-sprouting broccoli to be the result of the presence of anthocyanins.

Broccoli has also been found to exhibit antioxidant activity that prevents oxidative stress related to many diseases (Borowski, Szajdek, Borowska, Ciska and Zieliński, 2008).Currently, the use of broccoli by-products such as leaves and stems is restricted to flour and fiber Campas, Sánche, Bueno, Ramíre and López (2010), but the potential use of these by-products as important sources of phytochemicals is now gaining more attention in the scientific community (Mahro and Timm, 2007). Many studies on broccoli have been performed on the antioxidant and anticancer activities of broccoli components, but most of the studies analyzed florets of different varieties (Farag and Abdel, 2010). Domínguez et al., (2010) determined the antioxidant activity of broccoli leaves extract using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging method, vitamin C and phenols were estimated with the Folin–Ciocalteu reagent, flavonoids were evaluated using colorimetric methods and anthocyanins were determined by a pH differential method. Results showed broccoli to be having good antioxidant activity.

These antioxidants have proved to be good for human health and also useful as food preservatives (Kroon and Williamson 1999).Among crops included into Brassica vegetables, broccoli has been the most exhaustively studied with regard to polyphenol composition. Numerous and recent studies have shown that this crop (leaves, florets and sprouts) contains a high antioxidant potential linked to a high level of phenolic compounds (Moreno et al., 2006). Heimler et al., (2006) compared the main phenolic compounds in several B. oleracea crops and stated that broccoli and kale varieties exhibit the highest content of both total phenolics and flavonoids. Kurilich et al., (1999) reported a similar rank on the basis of concentration and, therefore, they pointed out that the best sources of lipid soluble antioxidants are kale and broccoli. Podsedek (2007) did a review of several works about antioxidant potential in B. Oleracea crops, brussels sprouts, broccoli and red cabbage belong to the group of the ones having the highest antioxidant capacity whereas cabbage demonstrated a rather low antioxidant activity.

2.7.1.3Antimicrobial potential of broccoli

In the context of health-promoting foods, broccoli (Brassica oleracea) is also considered a source of bioactive phytochemicals (Moreno et al., 2006). In fact, the data available reveal broccoli to be a healthy food due to the beneficial biological effects of its phytochemicals (Vasanthi, Mukherjee and Das, 2009). Broccoli has antimicrobial and anticancer activities (Moreno et al., 2006). A study bySibi (2013) evaluated the antimicrobial potential of broccoli extracts against food borne bacteria with a view to exploring its potential application in food industries as botanical preservatives. Preliminary antibacterial studies of broccoli extracts demonstrated its broad activity against the food borne pathogens. Farzinebrahimi, Taha, Fadainasab and Mokhtari (2012) has reported the antibacterial activity of leaf extracts of broccoli against *Pseudomonas aeroginosa*. Further, owing to its strong antibacterial activity, bioactive compounds from broccoli (*Brassica oleracea* L.var. *italica*) have scope for the possible use in food industries to stay away from food borne pathogens.

According to a research by Ares, Nozal and Bernal (2013) broccoli showed potent antibacterial activity and the active compounds were isolated, instrumental analysis identified the compounds as isothiocyanate's. Some homologues of isothiocyanate's were also active against *Escherichia coli, Staphylococcus aureus* and *Chlamydia*. The antimicrobial potential of seven compounds isolated from broccoli was tested against the growth of Klebsiella pneumoniae, Aspergillus flavus, and Bacillus cereus. The well-known, syringic acid (0.5 mg/ml) completely inhibited the growth of Bacillus cereus. p-hydroxy benzoic and p-coumaric acid (0.3 mg/ml) completely inhibited the growth *E.coli* and Klebsiella pneumoniae.

In a study by Kaur, Kumar, Anil and Kapoor (2007) extracts of Brassica oleracea (Broccoli, Cauliflower, Cabbage, Brussels Sprouts and Red Cabbage), Raphanus sativus (radish) and Brassica rapa (Bok Choy) showed significant antimicrobial activity against selected strains of pathogen. Ethanol extract showed highest antimicrobial activity than methanol, chloroform and diethyl ether extracts. Dimayuga (1991), investigated antibacterial activity against food borne pathogen using extract of petroleum ether, chloroform, ethyl acetate, acetone, methanol and aqueous of broccoli and determined the Minimum inhibitory concentration (MIC) values to be approximately 10 - 320 μg ml-1.

2.7.2Moringa Oleifera

Moringa oleifera, also called the drumstick tree, is a tree that grows in the foothills of the Himalayas in northern India. It is also cultivated throughout Central and South America and Africa due to the ease with which it grows in tropical and sub-tropical environments. While *Moringa oleifera* remains relatively unknown in the West, it has developed a reputation in its native lands for its unusually high nutritional value. Indeed, health researchers have started to give it nicknames such as "The Miracle Tree" and "The Elixir of Long Life" due to its miraculous healing abilities (Fahey, 2005).

Nutritional analysis has shown that *Moringa oleifera* leaves are extremely nutritious. In fact, they contain larger amounts of several important nutrients than the common foods often associated with these nutrients (Cushine and Lamb, 2005). These include vitamin C, which fights a host of illnesses including colds and flu; vitamin A, which acts as a shield against eye disease, skin disease, heart ailments, diarrhoea, and many other diseases; calcium, which builds strong bones and teeth and helps prevent osteoporosis; potassium, which is essential for the functioning of the brain and nerves, and proteins, the basic building blocks of all our body cells (Mboto et al., 2009). Another important point is that *Moringa oleifera* leaves contain all of the essential amino acids, which are the building blocks of proteins. It is very rare for a vegetable to contain all of these amino acids, and *Moringa oleifera* contains these amino acids in a good proportion, so that they are very useful to our bodies. These leaves could be a great boon to people who do not get protein from meat.

2.7.2.1 Antioxidant activity *Moringa oleifera*

According to analysis, the powdered leaves of the *Moringa oleifera* tree (which is the way most people consume moringa) contains 46 types of antioxidants. One serving, in fact, contains 22 percent of our recommended daily intake (RDI) of vitamin C, one of the most important antioxidants on Earth, and a whopping 272 percent of our RDI of vitamin A (Chuang et al., 2007). Antioxidants help to neutralize the devastating impact of free radicals, thereby guarding us from cancer and degenerative diseases such as macular degeneration and cystic fibrosis (Dahot, 1998).

2.7.2.2 Antimicrobial potential *Moringa oleifera*

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and

Afghanistan which is widely used for treating bacterial infection, fungal infection, anti-inflammation, sexually-transmitted diseases, malnutrition and diarrhoea (Fahey,2005). *Moringa* species have long been recognized by folk medicine practitioners as having value in the treatment of tumors (Ramachandran, Peter, and Gopalakrishnan, 1980). A study by Dahot (1998) reported that *M. oleifera* water extracts had antimicrobial activity against *E. coli*, *S. aureus* and *B. Subtilis*. Yang et al., (2006) reported that the inclusion of *Moringa oleifera* leaf meal in Broiler feeds reduced the *E. coli* bacteria count in the ileum. In addition, *Moringa oleifera* leaf water extracts exhibited antimicrobial properties through the inhibition of the growth of *S. aureus* strains isolated from food and animal intestines (Yang et al., 2006).

The leaves of *Moringa oleifera* have also been known to contain a number of phytochemicals such as flavonods, saponins, tannins and other phenolic compounds that have antimicrobial activities (Sato et al., 2004). This would suggest that the antimicrobial activities of moringa could be attributed to such compounds. The mechanisms of actions of these compounds have been proven to be via cell membranes perturbations (Esimone, Iroha, Ibezim, Okeh and Okpana , 2006). This coupled with the action of β-lactams on the trans-peptidation of the cell wall could lead to an enhanced antimicrobial effect of the combinations (Esimone et al., 2006). According to Dahot (1998), *Moringa oleifera* leaf extracts contain small peptides which could play an important role in the plant's antimicrobial defence system. The proteins or peptides are believed to be involved in a defines mechanism against phytopathogenic fungi by inhibiting the growth of micro-organisms through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Chuang et al., 2007).

2.8 PREVIOUS USE OF NATURAL PRESERVATIVES IN MEAT

Natural substances with antimicrobial action have been identified from a very wide range of sources including herbs and other edible and medicinal plants, microorganisms and animals. Many of these have been investigated but a few have been exploited as food preservatives on a commercial basis (Valko, Rhodes and Moncol, 2006). Plant polyphenol extracts have been used as natural meat preservatives including extracts from oregano, cranberry, sage, grape seed, rosemary and thyme. They are used as preservatives due to the presence of essential oils derived from these plants that contain most of their antimicrobial activity and they contain a variety of individual components that seem to be able to kill or inhibit the growth of microorganisms (Yang et al., 2006). Polyphenols can act as reducing agents and metal ion chelators in the presence of various hydroxyl radicals (Cushine and Lamb, 2005). Salt has also been used as a

natural preservative in meat since ancient times. Salt helps dehydrate microbes through the process osmosis and halts the growth of bacteria. Lemon juice has also been used as it contains plenty of vitamin C, which is a powerful antioxidant that prevents spoilage and rotting of meat

2.9 CONCLUSION

Chemical preservatives have side effects related to the emergence of drug resistant strains and chronic toxicity. Traditional methods of preservation including refrigeration, pasteurization and low pH are not completely effective in controlling food pathogens. Therefore the efficiency of using natural preservatives as antimicrobials should be tested.

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 RAW MATERIALS

Minced meat

Economy silverside beef steak was purchased from a butchery in Gweru. The beef steak was minced and put in seven different trays and stored in a refrigerator at 4^{o}C, before microbial, colour and sensory analysis was done.

*Brassica oleracea*L. var. *Italic*(Broccoli)

Broccoli leaves were collected from a garden in Mkoba, Gweru and were put in a zip bag. They were kept at room temperature (22^{o}C) before the extraction process.

Moringa oleifera

The *Moringa oleifera* leaves were obtained from a tree grown in Chegutu, Zimbabwe.

3.2 RESEARCH DESIGN

An experimental design was used for the research. Seven minced meat samples were prepared and preserved differently. They were tested for microbiological quality (Total Bacterial Count, Coliforms, *Salmonella, S. aureus* and *Escherichia coli*), colour stability and sensory evaluation. The analysis was carried out at 1 hour after production, after 12 hours, 24 hours, 48hours and a maximum of 72 hours.

3.2.1Experimental design

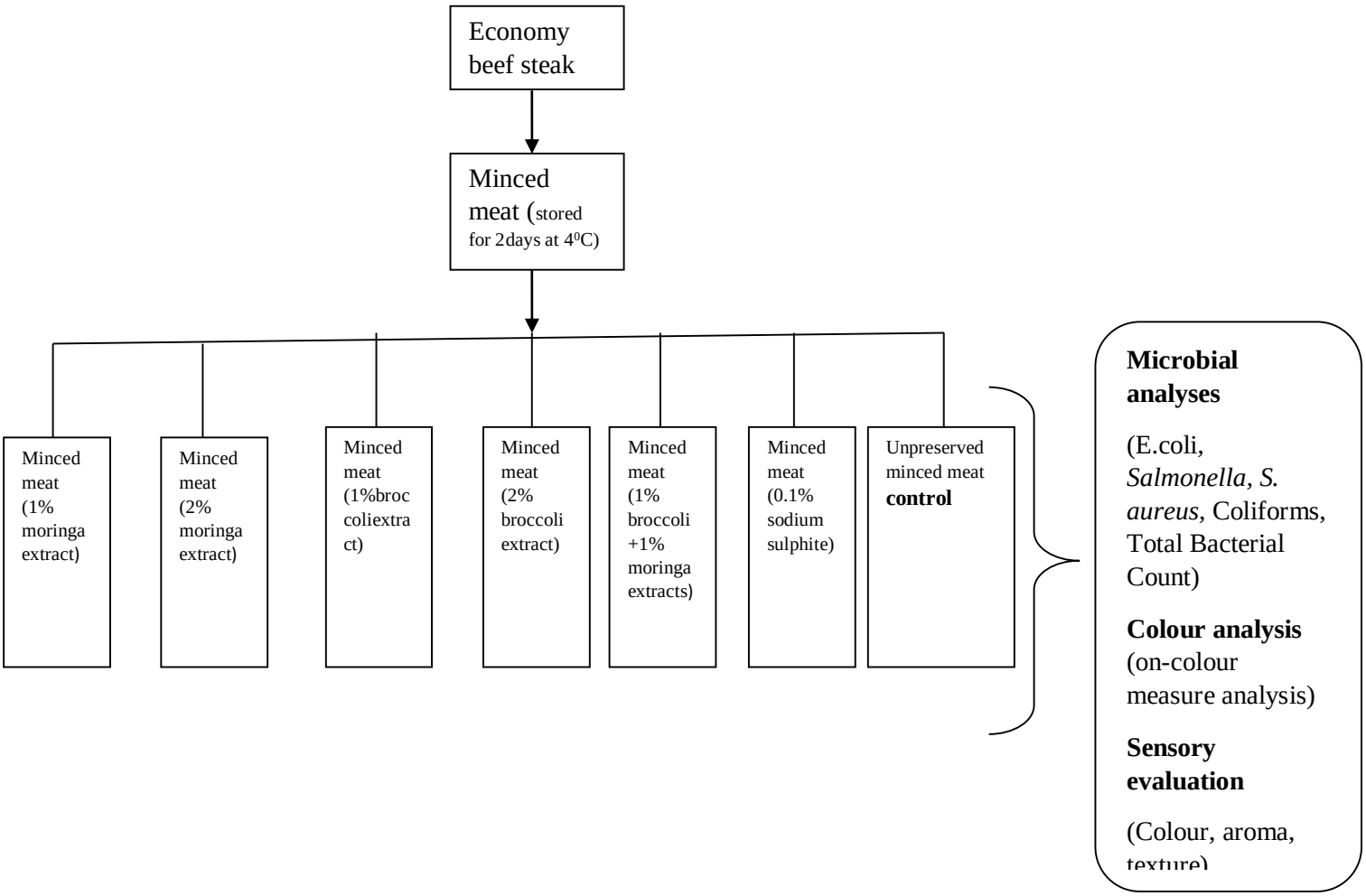


Fig 3.1: Experimental design (showing the control, sodium sulphite and various concentrations of *Moringa oleifera* and broccoli leaf extract)

3.3 METHODS OF EXTRACTION

3.2.1 Preparation and extraction of the *Moringa oleifera* leaf Extract

The extraction was prepared using the method by (Redfern, Kinninmonth, Burdass and Verran, 2014). The *Moringa oleifera* leaves were washed to remove any dirt and other impurities, they were then dried in open air until they reached constant weight. Soxhlet method using ethanol as a solvent was used to obtain the moringa extract. Dried leaves (50 grams) were first ground using a motor and pestle.

The ground leaves were wrapped by a filter paper and dampened with the ethanol then loaded into the thimble, which is placed inside the Soxhlet extractor. Ethanol was heated to 70^{o}C using a water bath in order for it to evaporate, that is moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reached the siphon it poured back into the flask and the cycle was repeated for 12 hours. The extracts were concentrated by evaporating solvent using water bath at 60°C and then left to dry.

3.2.2 Preparation of the Broccoli leaf extract

Mature leaves of broccoli were collected from a local market and brought to the laboratory in sterile zip lock bags for further investigation. The leaves were washed and dried at 22^{o}C. The dried leaves were ground into powder using a motor and pestle and put in a glass bottle containing 50ml of ethanol. The bottle was closed and kept in rotary shaker at 100rpm for 2 days to enhance proper dissolution of the bioactive compounds in the broccoli powder. The sample was then filtered at room temperature with a filter paper and the filtrate was then evaporated at 45^{o}C in a rotary evaporator until the extract becomes concentrated. The extract was stored in a refrigerator at 4^{o}C until analysis (Kunin 1983).

3.4 MICROBIAL ANALYSIS

3.4.1 Total bacterial count

One gram of each minced meat sample was carefully weighed and mixed with 9 ml of the peptone water. Further serial dilutions were prepared up to 10^{-10} and one ml of this dilution was plated by the pour plate method and using Nutrient Agar. The inoculated plates were incubated at 37^{0}C for 24 hours to obtain total viable count. Colonies were counted using the colon counter (Harrigan, 1998).

3.4.2 Coliforms

Coliforms are generally harmless but however it is a utility hygiene indicator test. One gram of each minced meat sample was weighed and mixed with 9ml of peptone water. Serial dilution was prepared to 10^{-5} and the Violet Red Bile Agar was used for enumeration of coliforms using the pour plate method and incubated at 37^{o}C for 24 hours (Harrigan, 1998).

3.4.3 E. coli

Enumeration of *E. coli* was done on Eosine Methylene Blue Agar (EMB) after incubation at 36^0C for 24 hours. MacConkey Broth was used for selective enrichment at 44ºC for 24 hours and typical *E. coli* colonies had a metallic green sheen on EMB.

3.4.4 Staphylococcus aureus

Staphylococcus aureus is a bacterial pathogen causing staphylococcal food poisoning. It was enumerated by using the spread plate method on a pre-dried surface of Baird-Parker agar and the plates were incubated at 37 °C for 24 hours. The serial dilution of 10^{-5} was used. *Staphylococcus aureus* typically forms colonies that are 1.0–1.5 mm in diameter, black, shiny, convex with a narrow white entire margin and surrounded by clear zones extending 2–5 mm into the opaque medium (Harrigan, 1998).

3.4.5 Salmonella

Salmonella is a life threatening bacterium and it is the major cause of most food borne bacterial illness in humans, (Hensel, 2004). Detection and enumeration of *Salmonella* colonies was done using Xylose Lysine Desoxycholate (XLD) Agar, and the plates were incubated at 36^0C for 24 hours. *Salmonella* enrichment broth was used for selective enrichment so as to encourage multiplication of *Salmonella* while inhibiting growth of competitive flora such as coliforms. Detection was done following the modified WHO Global Food borne Infections Network Laboratory protocol based on ISO 6579:2002.

3.5 COLOUR STABILITY

During the 72 hour storage period each minced meat sample was measured for colour using colour analysis software by research lab tools to determine the effects of preservative type on colour stability.

3.6 SENSORY ANALYSIS

For sensory analysis, unpreserved minced meat and the ones preserved with *Moringa oleifera* and Broccoli leaf extracts as well as sodium sulphite were boiled for 12minutes and kept warm in stainless steel containers. A 12 member consumer panel of students and staff from the Food science department at Midlands State University was used to taste and evaluate giving their opinion on the cooked mince from the four treatments. The minced meat samples were coded

with randomized letters and rotated to prevent bias. Chilled water was provided as a palette cleanser. The questionnaire below was used. The results were analysed using Xlstat software.

Sensory evaluation form

Ground Beef Sensory Evaluation Form; Name_____Date_____

Sample	Taste	Aroma	Texture
A			
B			
C			
D			
E			
F			
G			

Rating scale 1-5

1-like extremely **2**-like moderately **3**-niether like nor dislike

4- dislike moderately **5**- dislike extremely

3.7 DATA PRESENTATION AND STATISTICAL ANALYSIS

Graphs and tables were used to present the data. Graph Pad Prism 4 was used to analyze data using statistical tests which in this case was one way ANOVA. All the sensory analysis data was collected in spread sheets using Microsoft Excel and analysed using Xlstat software. Differences were considered significant at the $p < 0.05$ level.

3.8 VALIDITY AND RELIABILITY

The analyses were repeated at least twice and equipment used was calibrated and standardized. Standard methods were used in all analysis done.

CHAPTER FOUR: DATA PRESENTATION AND DISCUSSION

4.1 INTRODUCTION

The study was aimed at assessing the effectiveness of *Moringa oleifera* and broccoli leaf extracts as natural preservatives in minced meat comparing to minced meat preserved with sodium sulphite. This chapter presents and analyses the results found from microbiological, colour and sensory tests.

4.2 RESULTS

Total Bacterial Count

Table 1: Effects of *Moringa oleifera* and broccoli as preservatives on TBC of minced meat over a 72 hour storage period at 4°C

Preservative	1 hour	12 hours	24 hours	48 hours	72 hours
1%M	4.48	4.7	4.9	5.1	5.4
2%M	4.45	4.6	4.78	5	5.1
1%B	4.67	4.8	4.9	5.17	5.43
2%B	4.5	4.18	4.93	5.44	5.59
0.1%S	4.3	4.68	4.73	4.75	4.8
1%M +1%B	4.39	4.65	4.87	5.05	5.19
CONTROL	4.42	5.1	5.3	5.5	5.98

*M- moringa *B- broccoli *S- sodium sulphite

Generally as expected there was an increase in TBC of all minced meat samples with storage time. *Moringa oleifera* extract (1%) retarded the growth of microorganisms over time as compared to control minced meat without preservative as shown in table 2. Doubling the concentration of *Moringa oleifera* did not have much effect on TBC of minced meat. Furthermore, although both concentrations of *Moringa oleifera* had a low TBC than the control without preservatives, sodium sulphite had a better effect on TBC over time. As for broccoli like *Moringa oleifera* it retarded growth of micro-organisms overtime. Doubling the concentration of broccoli did not have much effect on TBC. Similarly to *Moringa oleifera* Sodium sulphite had a better effect on TBC as compared to broccoli. Overally the combination of *Moringa oleifera* and broccoli had a better effect on TBC as compared to the individual natural preservatives. However the number of TBC was higher as compared to sodium sulphate.

Coliforms

Table 2: Effects of *Moringa oleifera* and broccoli as preservatives on coliform count of minced meat over a 72 hour storage period at 4ºC

Preservative	1 hour	12 hours	24 hours	48 hours	72 hours
1%M	4.4	4.27	4.19	4.1	4.33
2%M	4.48	4.09	3.76	3.42	3.72
1%B	4.47	4.35	4.28	4.25	4.53
2%B	4.45	4.2	4.23	4.6	4.67
0.1%S	4.3	3.8	3.4	3.21	3.66
1%M +1%B	4.39	4	3.87	3.7	4
CONTROL	4.5	4.52	4.68	5	5.3

*M- moringa *B- broccoli *S- sodium sulphite

Generally there was a decrease in Total Coliform Count (TCC) on all the preserved minced meat samples with the exception of the control and broccoli. *Moringa oleifera* extract reduced the number of coliforms overtime as compared to the control which had an increase in the number of TCC with time. Increasing the concentration of *Moringa oleifera* extract to 2% resulted in a further decrease in the number of TCC. However, sodium sulphite had better preservative effect as compared to both concentrations of moringa. Doubling the concentration of broccoli did not have much effect on the total coliform count although the counts TCC of minced meat sample with 1% broccoli was lower than 2% broccoli. Comparing it to broccoli, sodium sulphite had a better effect on TCC. The effectiveness of the combination of *Moringa oleifera* and broccoli was as good as sodium sulphate, although the TCC count of sodium sulphite was slightly lower than the combination.

Staphylococcus aureus

Table 3: Effects of *Moringa oleifera* and broccoli as preservatives on *S. aureus* of minced meat over a 72 hour storage period at 4°C

Preservative	1 hour	12 hours	24 hours	48 hours	72 hours
1%M	2.27	2.5	1.88	2.2	2.6
2%M	2	1.97	1.32	2	2.51
1%B	2.01	2.47	2.81	2.89	3.77
2%B	2.4	2.7	2.82	2.39	3.58
0.1%S	1.6	2.47	1.58	1.77	2.53
1%M +1%B	1.81	2.46	1.4	2.21	2.73
CONTROL	2.42	2.9	3.2	3.62	3.91

*M- moringa *B- broccoli *S- sodium sulphite

Minced meat samples preserved with *Moringa oleifera* had a decrease in counts, and doubling the concentration of *Moringa oleifera* had a better preservative effect against *S. aureus*. The *S. aureus* counts of minced meat preserved with *Moringa oleifera* were higher than that of minced meat preserved with sodium sulphite but the difference was small. Minced meat preserved with broccoli had a gradual increase in counts of *S. aureus* over the 72 hour storage period. Doubling the concentration of the broccoli extract had little effect on the number of counts of *S. aureus*. Minced meat samples preserved with broccoli had higher *S. aureus* counts than the samples preserved with sodium sulphite. The *S. aureus* counts for minced meat preserved with broccoli were almost the same as with those of the unpreserved minced meat. Minced meat sample preserved with a combination of broccoli and *Moringa oleifera* had *S. aureus* counts which were quite similar to the minced meat sample preserved with sodium sulphite. *S. aureus* counts for minced meat preserved with a combination of broccoli and *Moringa oleifera* were low as compared to the control minced meat without preservative.

Colour a*-values (redness)

Table 4: Effects of *Moringa oleifera* and broccoli as preservatives on a*-values for minced meat over a 72 hour storage period at 4ºC

Preservative	1 hour	12 hours	24 hours	48 hours	72 hours
1%M	13.0	11.3	9.1	9.0	6.11
2%M	12.9	11.0	9.0	9.0	7.0
1%B	13.04	12.0	10.3	10	9.1
2%B	13.90	12.2	11.6	11	10.0
0.1%S	14.0	13.2	10.0	10	9.0
1%M +1%B	13.1	11.4	10.0	8.0	6.0
CONTROL	12.0	10.1	8.0	7.0	5.0

*M- moringa *B- broccoli *S- sodium sulphite

Table 5 shows the colour a* (stability of the red colour) values for the seven minced meat samples over the 72 hour period stored at 4 ºC. As expected, there was a reduction in the a*-values for all the samples. Minced meat samples preserved with *Moringa oleifera* had values lower than the values for minced meat samples preserved with sodium sulphite and broccoli, but higher than the control. Doubling the concentration of the *Moringa oleifera* extract had no effect on the a*-values. Minced meat samples preserved with broccoli had higher values than most of the minced meat samples. Broccoli had similar effect with sodium sulphite. Increasing the concentration of broccoli preservative had no effect in the a*-values, but minced meat samples preserved with 2% broccoli had values which were slightly higher than minced meat preserved with 1% broccoli. Minced meat preserved with a combination of broccoli and *Moringa oleifera* had higher values than minced meat preserved with moringa and the control, and the values were slightly lower than those of minced meat preserved with sodium sulphite.

Colour L*-values (lightness)

Table 5: Effects of *Moringa oleifera* and broccoli as preservatives on L*-values for minced meat over a 72 hour storage period at 4ºC

Preservative	1 hour	12 hours	24 hours	48 hours	72 hours
1%M	53.1	50.0	50.0	49.0	46.2
2%M	53.0	50.0	50.1	49.0	46.4
1%B	52.0	52.2	52.0	50.0	47.3
2%B	53.0	53.1	52.0	51.0	50.
0.1%S	53.2	52.0	50.7	51.0	50.8
1%M +1%B	52.0	51.0	48.8	48.1	47
CONTROL	51.0	50.0	48.1	48.0	44.2

*M- moringa *B- broccoli *S- sodium sulphite

Table 6 shows the colour L* (lightness) values for the seven minced meat samples over the 72 hour period stored at 4 ºC. There was no significant difference in the L*-values of all the minced meat samples. A slight reduction in all the counts was recorded as shown in table 6 above.

Colour b*-values (yellowness)

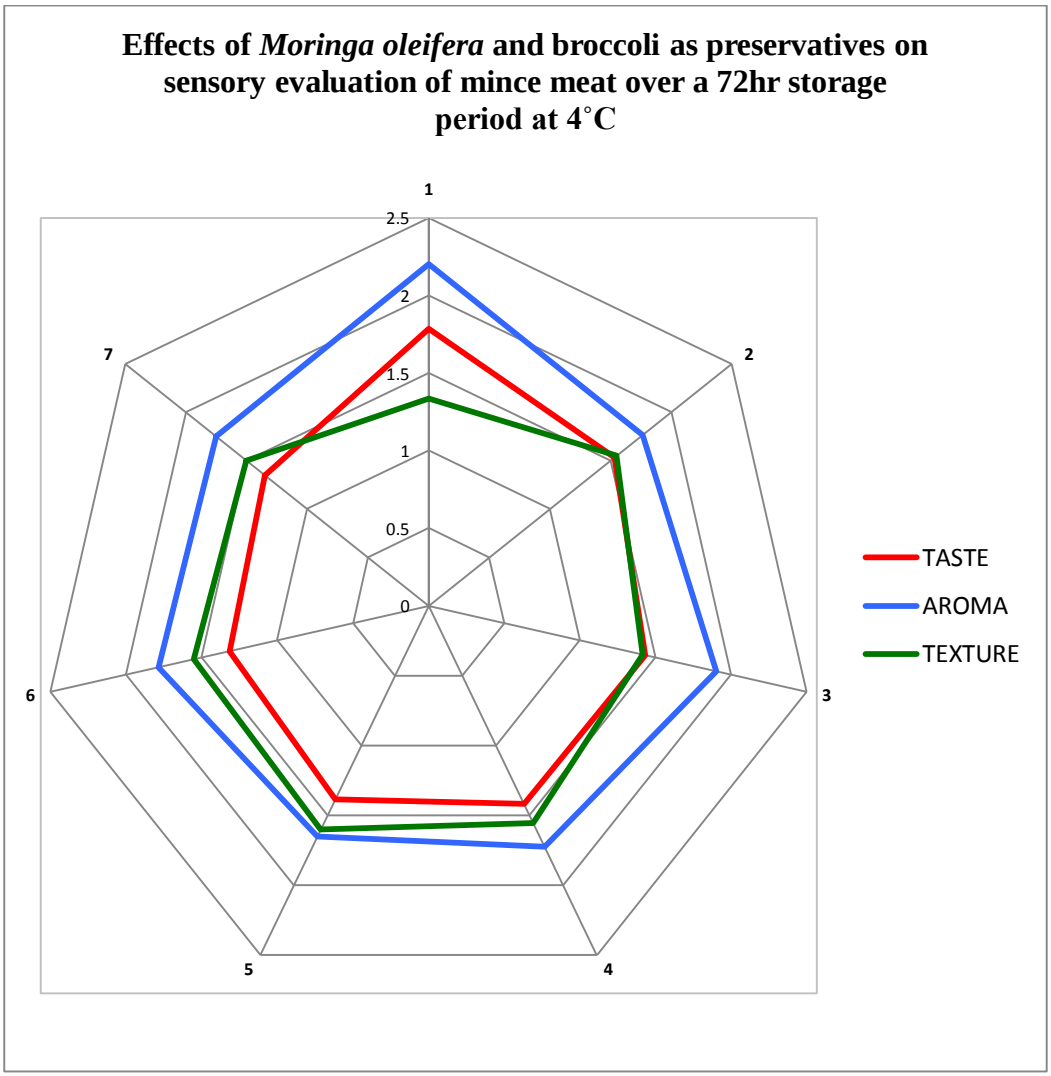
Table 6: Effects of *Moringa oleifera* and broccoli as preservatives on b*-values for minced meat over a 72 hour storage period at 4ºC

Preservative	1 hour	12 hours	24 hours	48 hours	72 hours
1%M	14	13	13	13	14
2%M	15	15	14	14	13
1%B	14	14	13	14	14
2%B	14	13	14	13	13
0.1%S	15	14	14	14	13
1%M +1%B	14	14	13	13	13
CONTROL	14	14	13	13	12

*M- moringa *B- broccoli *S- sodium sulphite

Table 7 shows the colour b* (yellowness) values for the seven minced meat samples over a 72 hour period stored at 4 ºC. There was a slight reduction of values with time on all the minced meat samples however, there were no significant difference between the values of the control or any of the samples.

Sensory evaluation



KEY

1-1% moringa **2**- 2% moringa **3**-1% broccoli **4**-2%broccoli
5-0.1**%** sodium sulphite **6**-1% moringa +1%broccoli **7**-control

Fig 4.1: Effects of *Moringa oleifera* and broccoli preservatives on the sensory evaluation of minced meat

Table 7: Xlstat Summary of the effects of *Moringa oleifera* and broccoli as preservatives on the sensory evaluation of minced meat

Summary (LS means) - SAMPLE:	SENSORY EVALUATION		
	TASTE	AROMA	TEXTURE
1% M	1.783 a	2.200 a	1.333 b
2%M	1.533 b	1.767 bc	1.550 ab
1%B	1.433 bc	1.900 b	1.417 ab
2%B	1.417 bc	1.722 bc	1.556 ab
0.1%S	1.383 bc	1.650 c	1.600 a
1%M+1%B	1.317 c	1.783 bc	1.550 ab
Control	1.350 bc	1.750 bc	1.500 ab
Pr> F	< 0.0001	0.000	0.247
Significant	Yes	Yes	No

*M-Moringa *B-Broccoli *S-sodium sulphite

Generally, there was no significance difference in the sensory scores of all the minced meat samples with the exception of minced meat preserved with *Moringa oleifera* which had higher scores on taste and texture. There was no significance difference between all the aroma scores of the minced meat samples.

4.3 HYPOTHESIS TESTING

Hypothesis 1

H_O1: There is no significant difference in the bacterial load (TBC) of minced meat preserved with *Moringa oleifera* and broccoli leaf extracts and the one preserved with sodium sulphite.

TBC one way ANOVA Summary of results using Graph Pad Prism 4 (*α* 0.05)

Table Analyzed			
TBC			
One-way analysis of variance			
P value	0.1989		
P value summary	Ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	7		
F	1.549		
R squared	0.2493		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	6.903		
P value	0.3299		
P value summary	Ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	Df	MS
Treatment (between columns)	1.160	6	0.1933
Residual (within columns)	3.494	28	0.1248
Total	4.654	34	

F calculated = 1.549

F critical = 2.61

Decision: Do not reject H_o

Conclusion: There is no significant difference in the bacterial load (TBC) of minced meat preserved with *Moringa oleifera* and broccoli leaf extracts and the one preserved with sodium sulphite.

H_O1: There is no significant difference in the bacterial load (coliforms) of minced meat preserved with *Moringa oleifera* leaf extract and the one preserved with sodium sulphite.

Coliforms one way ANOVA Summary of results using Graph Pad Prism 4 (α 0.05)

Table Analyzed			
Coliforms			
One-way analysis of variance			
P value	P<0.0001		
P value summary	***		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	7		
F	8.183		
R squared	0.6368		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	9.830		
P value	0.1320		
P value summary	Ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	Df	MS
Treatment (between columns)	4.004	6	0.6673
Residual (within columns)	2.283	28	0.08155
Total	6.287	34	

F calculated = 8.183

F tabulated = 5.19

Decision: Reject Ho

Conclusion: There is a significant difference in the total coliform countsof minced meat preserved with *Moringa oleifera* and broccoli leaf extracts and the one preserved with sodium sulphite.

H_O1: There is no significant difference in the bacterial load (*Staphylococcus aureus*) of minced meat preserved with *Moringa oleifera* and broccoli leaf extracts and the one preserved with sodium sulphite

Staphylococcus aureus **one way ANOVA Summary of results using Graph Pad Prism 4 (α 0.05)**

Table Analyzed			
Staph. aureus			
One-way analysis of variance			
P value	0.0017		
P value summary	**		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	7		
F	4.838		
R squared	0.5090		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	2.215		
P value	0.8989		
P value summary	Ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	Df	MS
Treatment (between columns)	6.602	6	1.100
Residual (within columns)	6.368	28	0.2274
Total	12.97	34	

F calculated = 4.838

F tabulated = 5.19

Decision: Reject Ho

Conclusion: There is a significant difference in thebacterial load (*Staphylococcus aureus*) minced meat preserved with *Moringa oleifera* and broccoli leaf extracts and the one preserved with sodium sulphite.

H_O2: There is no significant difference in the quality and shelf life of minced meat preserved with *Moringa oleifera* leaf extract and broccolileaf extract to the one preserved with sodium sulphite.

Colour a*-value one way ANOVA Summary of results using Graph Pad Prism 4 (α 0.05)

Table Analyzed			
colour a*-values (redness)			
One-way analysis of variance			
P value	0.3685		
P value summary	Ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	7		
F	1.134		
R squared	0.1956		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	2.386		
P value	0.8810		
P value summary	Ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	Df	MS
Treatment (between columns)	36.17	6	6.029
Residual (within columns)	148.8	28	5.314
Total	185.0	34	

F calculated = 1.134

F tabulated = 6.72

Decision: Fail to Reject H_o

Conclusion: There is no significant difference in the quality and shelf life of minced meat preserved with *Moringa oleifera* and broccoli leaf extracts to the one preserved with sodium sulphite.

Colour L*-value one way ANOVA Summary of results using Graph Pad Prism 4 (α 0.05)

Table Analyzed			
colour L*-value (lightness)			
One-way analysis of variance			
P value	0.1242		
P value summary	Ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	7		
F	1.856		
R squared	0.2845		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	5.538		
P value	0.4769		
P value summary	Ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	Df	MS
Treatment (between columns)	49.94	6	8.324
Residual (within columns)	125.6	28	4.486
Total	175.5	34	

Colour b*-Value one way ANOVA Summary of results using Graph Pad Prism 4 (α 0.05)

Table Analyzed			
colour b*-value (yellowness)			
One-way analysis of variance			
P value	0.0941		
P value summary	Ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	7		
F	2.035		
R squared	0.3037		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	4.970		
P value	0.5477		
P value summary	Ns		
Do the variances differ signif. (P < 0.05)	No		
ANOVA Table	SS	Df	MS
Treatment (between columns)	5.778	6	0.9630
Residual (within columns)	13.25	28	0.4732
Total	19.03	34	

4.4 DISCUSSION

Total Bacteria Count

The control sample without a preservative reached a value of over 10^5cfu/g after 72 hours of storage, and this is the arbitrary shelf life "end point" where signs associated with spoilage are found (Steyn, 1989). The minced meat samples with broccoli did have a preservative effect on total bacterial counts due to the presence of polyphenols, isothiocyanates and phytochemicals such as flavonoids, saponins and tannins that have antimicrobial activities (Sato et al., 2004). The antimicrobial activities of isothiocynates derived from *Brassicaceae* vegetables, such as cauliflower, broccoli, mustard, and cabbage are related to loss of cell membranes integrity, inhibiting enzyme or regulatory activity by quorum sensing, inhibition of respiratory enzymes, induction of heat-shock and oxidative stress, and induction of a stringent response (Bajpai et al., 2008).

Moringa oleifera had a good antimicrobial activity, this is in agreement with a research by Okorondu, Akujobi, Okorondu, & Anyado-nwadike, (2015) which states that the quantitative phytochemical screening of *Moringa oleifera* revealed the presence of flavonoids alkaloids, tannins, saponins and cyanogenic glycosides for bioactive compounds which in correct doses can successfully be used to inhibit and eventually destroy microorganisms. *Moringa oleifera* also has phenolic compounds which can act as reducing agents and metal ion chelators in the presence of various hydroxyl radicals (Dorman and Deans, 2000).

The combination of broccoli and *Moringa oleifera* produced a better antibacterial effect than their individual effects. This is in line with the work of Stanojevic, Comic, Stefanovic & Solujic-sukdolak (2009) who mention that there is effective antimicrobial action of preservatives when used in combination with other preservatives than when used individually hence it can contribute to more effective conservation of food. Sodium sulphite proved to be a better preservative as compared to all the other preservatives. The use of sodium sulphite as preservative has been widely documented. The legal amount of sodium sulphite allowable in fresh minced meat is 0.1% of the meat weight (Department of Health, 2001). Sodium sulphite is more effective against the growth of Gram-negative rods, such as *E. coli* and *Pseudomonas*, than in inhibiting Gram-positive rods, such as *Lactobacillus* (Ough, 1993).

Coliforms

There was a gradual increase in the total coliform count of the control over the 72 hours storage period. There was an increase in the total coliform counts of all the samples at 72 hours suggesting that the preservatives could not work longer than the 48 hours which is the shelf life of minced meat. *Moringa oleifera* as a preservative had a good effect against coliforms; this is probably because of the phenolic compounds present in *Moringa oleifera*. The high antibacterial activity of phenolic compounds can be due to alkyl substitution into the phenol nucleus, forming phenoxy radicals which do not occur in more stable molecules such as the ethers myristicin or anethole (Dorman and Deans, 2000). Minced meat sample preserved with broccoli had significantly higher coliform counts than the minced meat samples preserved with sodium sulphite suggesting that broccoli had a bacteriostatic effect whereas *Moringa oleifera*, sodium sulphite and a combination of broccoli and *Moringa oleifera* preservatives had bactericidal effects. The bactericidal effect of the combination of moringa and broccoli on coliforms, could suggest a synergistic working between these preservatives (Stanojevic, Comic, Stefanovic and Solujic-sukdolak, 2009).

Although the presence of *E. coli* was investigated in this study, it could not be detected in any of the samples. This could be ascribed to good manufacture hygiene. A study by Charimba et al., (2010) found a reduction in *E. Coli* counts after 2 days of storage at 4^{o}C both in the presence and absence of a preservative (450 mg/kg SO2) at both a high and low inoculum of *E. coli* into some boerewors model. It could also be a similar case as the study done by Roller et al.,(2002) they did a study to develop a novel preservation system for fresh pork sausages based on a combination of chitosan and low concentrations of sulphite. Their results suggested that selective inactivation and inhibition of Gram-negative bacteria had occurred. They did not do counts on *E. coli*, but only on Gram-negative bacteria as a group. They explained the efficacy of the chitosan/sulphite combination on the basis that chitosan protected sulphite from breakdown. Neall (2006) reported that *Moringa oleifera* has a broad spectrum antimicrobial activity, which works against most bacteria (Gram-positive and Gram-negative). Sodium sulphite is more effective against the growth of Gram-negative rods, such as *E. coli*, than in inhibiting Gram-positive rods. In *E. coli*, NAD-dependent formation of oxaloacetate from malate is inhibited (Ough, 1993).

Until recently, the use of sodium sulphite has had GRAS status. Investigations have indicated certain asthmatic individuals were placed at risk by relatively small amounts of sulphites

(Roller et al., 2002). This has caused a great deal of research in all areas concerning sulphites and SO2. Nadarajah et al.,(2005) examined allyl isothiocyanate (AIT) for its ability to reduce numbers of *E. coli* O157:H7 inoculated in fresh ground beef packaged under nitrogen and stored refrigerated or frozen. Mesophilic aerobic bacteria in ground beef patties were largely unaffected by the addition of AIT. An initial population of 3 log10 cfu/g *E. coli* was reduced by AIT to undetectable levels after 18 days at 4ºC or 10 days at -18ºC. Samples inoculated with 6 log10 cfu/g had a higher than 3 log10 reduction of *E. coli* O157:H7 after 21 days at 4 ºC, and a 1 log10 reduction after 8 days at 10 ºC and 35 days at -18 ºC.

S. aureus

Moringa oleifera proved to be good in the prevention of gram-positive bacteria. Increasing the concentration of *Moringa oleifera* had a better effect this is because, antimicrobial mechanisms of phenol compounds depend on their concentration. Phenols affect enzyme activity related to energy production at low concentrations, however they cause protein denaturation at high concentrations (Bajpai et al., 2008). It could be concluded that *Moringa oleifera* and broccoli could be a good combination in the prevention of Gram-positive bacteria in this type of meat product. Broccoli on its own did not act well against *S. aureus,* this maybe because the concentration of broccoli was low for it to act against *S. aureus.*

Other studies on natural preservatives to inhibit *S. aureus* have been done, for example Shan et al., (2009) did a study to find natural spice and herb extracts with antibacterial and antioxidant capacities that could potentially be used as natural preservatives in raw pork. The inhibitory effects of cinnamon stick, oregano, pomegranate peel and grape seed extracts on *Listeria monocytogenes, S.aureus* and *Salmonella Enterica* were evaluated in raw pork at room temperature (~ 20 ºC). The results showed that all five natural extracts, especially clove, were effective against the bacteria. The conclusion was made that the tested extracts, especially clove, have potential as natural preservatives to reduce the numbers of a pathogenic bacteria like *S. aureus*. *Salmonella* was investigated in this study and samples tested negative for it. This might be because the minced meat sample was kept at the right temperature and there was less movement of the sample.

Colour a*-values

Studies in meat colour often focus on a*- value (redness), because the redness of the meat is an important component of visual appeal to customers. Several authors have studied the colour of meat and meat products. They have reported that the meat oxidation caused a decrease in a* value which is normally unacceptable for consumers (Banon, 2007). Broccoli samples showed some good colour stability properties and this could be as a result of polyphenols, a large group of phytochemicals found in broccoli that are often considered the most abundant antioxidants in the diet (Faller and Fialho, 2009). *Moringa oleifera* on its own did not have good colour stability properties but the combination of *Moringa oleifera* and broccoli produced good results. This could be as a result of antioxidant cycling which is a term that describes how antioxidants work together to extend each other's life and making each other more powerful (Banon, 2007).

Sodium sulphite is well known as antioxidant. Other materials can act as antimicrobial agents, but none has been found to replace the antioxidant capabilities of sodium sulphite (Ough, 1993). The preservation of the colour and odour of meats are improved by sulphite treatment. Although slowing or prevention of growth of surface bacteria is probably important, the main effect in meat appears to be the antioxidant properties (Ough, 1993).

Colour L*-values (lightness)

It could be concluded that the L*-value was relatively stable in all the treatments. The L*-value was done to determine the "lightness" of the colour of the product, the higher the value the lighter the product, a value of 100 = white and a value of 0 = black (Shan et al., 2009).Similar results were found in the mentioned study of Banon et al., (2007) on green tea (GTE) and grape seed (GSE) extracts as preservatives of low sulphite raw beef patties, they found the L* value was quite stable throughout storage in all patty groups. The extract addition did not affect L*, differences in mean L* between treatments were not significant ($p < 0.05$).

Colour b* values

In general the values were quite constant. The b* value is indicative of the yellowness of the colour of the product, a lower value is preferred, so that it does not affect the redness of the product.

Sensory evaluation

A five point hedonic scale was used. After 72 hours the taste aroma and texture of the minced meat samples was still acceptable. There was no development of any off smells or flavours in all the samples. One of the general remarks was that the panellists could not tell any difference in taste among all the minced meat samples. Similar results were found in the following studies. Kanatt *et al.* (2008) found that at a 0.1% addition of a chitosan and mint (CM)mixture in pork cocktail salamis, the initial sensory analysis showed that there was no significant ($p < 0.05$) difference between the treated and untreated samples. With respect to colour, flavour, taste and texture, the CM-treated and control samples were similar.

Banon *et al.* (2007) found that green tea extract (GTE) and grape seed extract (GSE) in combination with low sulphite concentrations did not produce appreciable odour, flavour or texture in cooked beef patties.

CHAPTER FIVE: SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 SUMMARY

The purpose of the study was to determine the antimicrobial efficacy of *Moringa oleifera* and broccoli leaf extracts as natural preservatives in minced meat over 72 hours of storage at 4^{o}C. Seven minced meat samples were prepared with different preservative concentrations and combinations (1% moringa, 2% moringa, 1% broccoli, 2% broccoli, 0.1% sodium sulphite and a combination of 1% moringa and 1% broccoli). Observations as presented in Chapter 4 revealed that *Moringa oleifera* did have a preservative effect on the microbial load of the minced meat. Antibacterial activity of broccoli in the minced meat was poor as compared to *Moringa oleifera* and sodium sulphite. Colour was measured using on-colour analysis software. The evaluation of *Moringa oleifera* on the colour of the minced meat showed a negative effect, especially on the colour a* (redness) value. However, broccoli had a great impact on the colour of minced meat as shown by the high values the colour a*-value. The combination of *Moringa oleifera* and broccoli preservatives produced a good result on the colour and microbial tests of the minced meat compared to individual natural preservatives, but it was not better than the sodium sulphite preservative. *Salmonella* spp. and *E.coli* were not detected in all the samples of minced meat. The texture, aroma and taste of the minced meat were good on all the samples throughout the storage period. There was no significance difference in the sensory attributes of minced meat preserved with *Moringa oleifera* and broccoli as compared to the minced meat preserved with sodium sulphite.

5.2 CONCLUSION

From this study, *Moringa oleifera* did preserve against total bacterial counts, coliforms and *S. aureus*. Although broccoli did not show much antimicrobial effect, it has good antioxidant properties. The combination of *Moringa oleifera* and broccoli appears to have a better preservative effect as compared to the individual natural preservatives. Furthermore the combination of the two natural preservatives produced a better colour than the individual preservatives. However, the combination of *Moringa oleifera* and broccoli in this study were not as effective as sodium sulphite. The results suggest that the combination of the natural preservatives rather than the individual are potentially more effective to achieve the effect as same as synthetic preservatives.

5.3 RECOMMENDATIONS

- Various plant preservatives should be tested in combination with *Moringa oleifera* to investigate the synergistic effect of the plant preservatives.
- Another study should be done using different extraction methods of *Moringa oleifera* and increasing the *Moringa oleifera* concentration.
- The preservative effect of *Moringa oleifera* should be analysed in different food products.

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