Sokoine University of Agriculture

MSc. Dissertation

Potential of Moringa Oleifera Leaf Extract in Ground Beef Preservation

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POTENTIAL OF MORINGA OLEIFERA LEAF EXTRACT IN GROUND BEEF PRESERVATION

Dissertation submitted to Sokoine University of Agriculture in Fulfillment of the Requirements for Master Degree in Food Quality and Safety Assurance

By

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EXTENDED ABSTRACT

This study explored the diverse potential of *Moringa oleifera* leaf extract as a natural preservative in ground beef, with a specific focus on its impact on microbiological and physico-chemical properties during refrigerated storage. The study explored varying concentrations of *Moringa oleifera* (0.5%, 1%, 1.5% and 2.0%) in comparison to a preservative-free control group, investigating their impact on microbial parameters, including Total Bacteria Counts (TBC), Total Coliform Count (TCC), *Staphylococcus, Salmonella*, and *E. coli*. The results unveiled a dose-dependent relationship, where in all concentrations exhibited lower TBC compared to the control group, with the 2.0% concentration demonstrating the most substantial reduction, underscoring *Moringa oleifera'*s potential to inhibit bacterial growth in ground beef.

In terms of TCC, higher concentrations of *Moringa oleifera* showed enhanced effectiveness in reducing coliform bacteria, particularly the 1.5% and 2.0% concentrations, which significantly decreased counts by 31% and 32% compared to the control group. The study also spotlighted *Moringa oleifera's* dose-dependent antibacterial activity against *Staphylococcus* and *E. coli*, with escalating doses correlating with significant reductions in these microorganisms. These findings collectively suggest that *Moringa oleifera* holds promise in augmenting food safety by curbing the proliferation of harmful bacteria, thereby acting as an effective natural preservative and enhancing the microbiological quality of refrigerated ground beef.

The investigation extended to the antimicrobial effects of *Moringa oleifera* aqueous extract on ground beef, assessing physico-chemical, microbial, and organoleptic qualities during refrigerated storage at 4°C. The results revealed that the 1.5% crude extract significantly improved ($p<0.05$) various sensory attributes, including pH, juiciness, texture, flavor, taste, and overall acceptability, surpassing both the control and other treated samples. Furthermore, microbial load, as indicated by the Aerobic Plate Count (APC), exhibited a significant decrease in treated samples ($p<0.05$), with the 2% treatment proving to be the most effective.

The color parameters (L*, a*, b*) of ground beef were influenced by *Moringa oleifera* extract, manifesting a decrease in values, particularly with the 2% concentration. Notably, the pH of ground beef experienced a slight increase during storage, but Moringa extract did not significantly ($p>0.05$) impact this parameter. In conclusion, this study underscores the potential of natural additives such as *Moringa oleifera* to not only extend the shelf life of ground beef but also to significantly enhance its microbiological and sensory properties. This aligns with the escalating consumer preference for safe and natural food products, emphasizing the need for further exploration and application of *Moringa oleifera* in the realm of food preservation and safety.

IKIRISI KUU

Utafiti huu unachunguza uwezo mbalimbali wa *Moringa oleifera* kama mchanganuo wa asili kwenye nyama ya ng'ombe, ukilenga athari zake za kibayolojia na kikemia wakati wa kuhifadhi kwenye baridi. Utafiti huu uliangazia viwango tofauti vya *Moringa oleifera* (0.5%, 1%, 1.5%, na 2.0%) ikilinganishwa na kundi la kudhibiti bila kemikali yoyote ya kuhifadhia, uchunguzi ukilenga athari zake kwenye viashiria vya kibayolojia, ikiwa ni pamoja na Jumla ya Idadi ya Bakteria (TBC), Idadi ya *Coliform* Jumla (TCC), *Staphylococcus*, *Salmonella*, na *E. coli.* Matokeo yalifunua uhusiano unaotegemea dozi kati ya viwango vya *Moringa oleifera* na idadi ya bakteria, na viwango vyote vikionyesha TBC ya chini ikilinganishwa na kundi la kudhibiti. Kwa kiasi kikubwa, kiasi kikubwa zaidi (2.0%) kilionyesha kupungua kwa kiasi kikubwa zaidi, kuthibitisha uwezo wa *Moringa oleifera* kuzuia ukuaji wa bakteria kwenye nyama ya ng'ombe.

Kuhusu TCC, viwango vya juu vya *Moringa oleifera* viliashiria ufanisi mkubwa katika kupunguza bakteria aina ya Coliform, haswa viwango vya 1.5% na 2.0%, ambavyo vilipunguza kwa kiasi kikubwa ikilinganishwa na kundi la kudhibiti. Utafiti pia ulitilia mkazo shughuli ya antibakteria ya *Moringa oleifera* inayotegemea dozi dhidi ya Staphylococcus na E. coli, na viwango vikiongezeka kulingana na kupungua kwa kiasi kikubwa kwa viumbe hawa vidudu. Matokeo haya yanahusisha kuwa *Moringa oleifera* inaahidi kuimarisha usalama wa chakula kwa kuzuia kuenea kwa bakteria hatari, hivyo kufanya kama kichocheo cha asilia cha kuhifadhi na kuboresha sifa za kibayolojia kwenye nyama ya ng'ombe iliyohifadhiwa kwenye baridi.

Uchunguzi huu ulienea hadi kwenye athari za antimikrobiolojia za dondoo ya maji ya *Moringa oleifera* kwenye nyama ya ng'ombe, ukichunguza sifa za kikemia, kibayolojia, na hisia za kuonja wakati wa kuhifadhi kwenye baridi ya nyuzi 4°C. Matokeo yalionyesha kuwa dondoo ghafi ya 1.5% iliboresha kwa kiasi kikubwa sifa mbalimbali za hisia, ikiwa ni pamoja na pH, utamu, muundo, ladha, harufu, na kukubalika kwa ujumla, ikizidi kundi la kudhibiti na sampuli zingine zilizosindikwa. Aidha, mzigo wa vijidudu, uliopimwa kama Jumla ya Viumbe vya Bakteria (APC), ulipungua kwa kiasi kikubwa katika sampuli zilizosindikwa, na matibabu ya 2% yakionyesha ufanisi zaidi.

Vigezo vya rangi (L*, a*, b*) vya nyama ya ng'ombe vilichochea na dondoo ya *Moringa oleifera*, ikionyesha kupungua kwa thamani, hasa na kiasi cha 2%. Kwa mujibu wa utafiti, pH ya nyama ya ng'ombe ilionyesha ongezeko dogo wakati wa kuhifadhi, lakini dondoo ya Moringa haikuathiri kwa kiasi kikubwa parameter hii. Kwa ujumla, utafiti huu unasisitiza uwezo wa viungo asilia kama *Moringa oleifera* si tu kuongeza muda wa kuhifadhi wa nyama ya ng'ombe, bali pia kuimarisha sana sifa zake za kibayolojia na hisia. Hii inakwenda sambamba na upendeleo unaoongezeka wa watumiaji kwa bidhaa za chakula zenye usalama na asilia, hivyo kutia mkazo umuhimu wa kuendelea kuchunguza na kutumia *Moringa oleifera* katika uga wa uhifadhi na usalama wa chakula.

DECLARATION

I, **Lucy J. Mwankunda**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

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Dr. Frida A. Nyamete Date

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Dr. Beatrice M. Kilima Date

(Supervisor)

LIST OF PUBLISHED PAPERS

1. Mwankunda, L. J., Nyamete, F., & Kilima, B. (2023). Exploring the Influence of Moringa oleifera Leaves Extract on the Shelf Life of Ground Beef during Refrigerated Storage.*EuropeanJournalofMedicinalPlants*,*34*(10),42-52.https://doi.org/10.9734/ejmp/2023/v34i101164

2. Mwankunda, L. J., Nyamete, F., Kilima, B. (2024). Antimicrobial Effect of Moringa oleifera Leaves Extract on Foodborne Pathogens in Ground Beef. International Journal of Microbiology and Biotechnology, 9(1), 15-20. https://doi.org/10.11648/ijmb.20240901.13

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DEDICATION

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LIST OF ABREVIATIONS AND ACRONYMS

CFU	Coliform forming unit
Color a*	Redness
Color b*	Brightness
Color l*	Yellowness
FAO	Food Agriculture Organization
ISO	International Organization for Standard
ML	Milliliter
MLE	Moringa Leaf Extract
MOLE	*Moringa Oleifera* Leaf Extract
SD	Standard deviation
SEs	Staphylococcal enterotoxins
SFP	Staphylococcal food poisoning
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau Standard
TCC	Total Coliform Count
TVC	Total Viable Count
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Ground beef refers to beef that has undergone the process of grinding or coarsely cutting through the use of a meat grinder or chopping knife. The process of grinding meat is known to enhance the tenderness of tougher cuts of beef. Additionally, incorporating fatty cuts into lean cuts during the grinding process has been shown to mitigate dryness and enhance the flavor profile of typically less succulent cuts. According to Wang *et al.* (2017), ground beef possesses a higher perishability compared to full-muscle cuts of meat, necessitating careful handling.

Moreover, in the event that any bacteria are present on the surface, the act of grinding disseminates them uniformly throughout the product, giving rise to food safety concerns specific to ground beef, which are not applicable to entire slices of meat. According to Salim *et al.*, (2018), the inside portion of an animal's flesh is inherently sterile prior to cooking, implying that any bacterial contamination is primarily confined to the meat's external surface (Vanderlinde et al., 2022). If ground beef is not cooked thoroughly, there is a high likelihood that a sufficient number of pathogenic bacteria will remain viable and could lead to disease. Furthermore, it is important to note that the process of warming can expedite the reproduction of bacteria. Therefore, it is imperative to ensure the appropriate preservation of ground meat (Kim *et al*., 2013).

1.2 Shelf Life of Ground Beef

Shelf life is defined as the time between a product's packaging and its final use during which the product's properties remain acceptable to the product user. Appearance, texture, flavor, color, and nutritive value are all examples of shelf-life properties (Nikmaram *et al.,* 2018). When determining the shelf-life of a meat product, such as ground beef, some people distinguish between case-life and shelf-life. Case-life (also known as color shelf-life or display-life) refers to the amount of time meat can be displayed under refrigeration before changing color. This color shift from bright cherry-red beef to another color, such as brown, is caused by an oxidation-induced change in the protein myoglobin (Salim *et al.,* 2018).

The change in color isn't harmful or doesn't make the food bad, but rather make the food colour undesirable to consumer. Shelf life is the length of time that a commodity may be stored without becoming unfit for use, consumption, or sale. The meat industry works hard to prevent, reduce, and get rid of both harmful and spoilage bacteria before delivered to selling points or consumers. Fresh ground beef can be kept in a refrigerator at 4°C up to two days (Bahmid *et al.,* 2021).

Meat is a nutrient-dense, protein-rich food that is extremely perishable and has a short shelf life if not properly preserved. It is the primary choice of animal protein for a significant portion of the global population. The global consumption of beef is steadily rising (Marinova & Bogueva, 2019). Spoilage of ground beef can be viewed as an ecological event involving the transformation of accessible components during bacterial proliferation in the microbial association of preserved meat (Furbek, 2022).

Microorganisms, lipid oxidation, and autolytic enzymatic spoiling are the primary causes of ground beef decomposition after slaughtering, during processing and storage. For meat to be transported without losing its texture, color, or nutritional content, it must be preserved. The term "minced beef deterioration" refers to any change that renders the product unfit for human consumption (Rahman *et al.,* 2023). Microbial proliferation produces unacceptable color flaws, textural alterations, the development of off-flavor, off-odor, and slime in meat.

1.4 Pathogenic Bacteria in Meat

Pathogenic bacteria are bacteria that have the ability to cause disease. Humans are generally most interested in bacteria that can cause disease in humans, despite the fact that these bacteria can also infect other animals and plants. Among the many pathogenic bacteria are *Streptococcus, Staphylococcus, Salmonella*, and *Escherichia coli.* The majority of food-borne illnesses are caused by pathogenic microorganisms in the environment (Abebe *et al.,* 2020).

1.4.1 *Escherichia coli*

E. coli is a gram-negative, short rod-shaped, non-spore-forming bacteria capable of growth and gas production at 45.5C. Strains of *E. coli* are innocuous occupants of the human and animal gastrointestinal tract. However, numerous food-borne pathogenic *E. coli* strains are known to exist (Hassan *et al.,* 2021). After implanting and colonizing the colon, *E. coli* O157:H7 and other enterohemorrhagic E. coli create a toxin(s), resulting in sickness. This bacterium is considered a "toxico-infectious" agent, as opposed to an invasive pathogen, because preformed toxins have not been proved to present in foods or to cause human disease (such as *Salmonella spp.*). However, there have been reports of evidence for an intrusive process (Wasiński, 2019). It is a challenging organism to handle from a public health perspective because to its low infectious dose, which may be partially attributable to its high acid tolerance and its ability to withstand low pH levels occasionally encountered in the stomach (Gambushe *et al.,* 2020). When beef is slaughtered and processed, *E.coli* bacteria in the cows' intestines can transfer to the flesh, contaminating ground beef with *E.coli.*

1.4.2 *Salmonella*

Salmonella species are gram-negative, rod-shaped, typically motile, Enterobacteraceae family members. Despite significant breakthroughs in molecular genetic techniques to identification and characterization, these organisms are still characterized serologically, by their somatic (O), flagellar (H), and occasionally capsular (Vi) antigens (Zahra *et al.,* 2023). Approximately 2,400 distinct serotypes have been identified. This microbe's nomenclature has undergone several revisions, resulting in considerable confusion. *Salmonella* is a life-threatening bacterium and a major source of food-borne bacterial infection in humans. *Salmonella* is the second most prevalent bacterial cause of food-borne gastroenteritis worldwide, after *Campylobacter* (Thames & Theradiyil, 2020). Salmonella can induce a variety of illness syndromes, such as typhoid fever caused by Salmonella typhi. Other *Salmonella* strains cause gastroenteritis, bacteraemia, and enteric or paratyphoid fever (Gast and Porter, 2020). *Salmonella* is a facultative aerobe, meaning it can grow in settings high in oxygen. When *Salmonella* is exposed to meat, it is able to grow. This exposure is caused by contact with feces, followed by handling of meat.

1.4.3 *Staphylococcus aureus*

Staphylococci are classified under the genus *Micrococca* and subfamily *Coccidae*. They're spherical, gram-positive bacteria that cluster like grapes and measure around 1 m in diameter. As staphylococci only divide in one plane, their grape-like shape sets them apart from streptococci, which cluster together (Milami *et al.,* 2023). *Staphylococcus aureus* (*S. aureus*) is a prevalent bacterial infection that causes staphylococcal food poisoning (SFP), which is a prominent cause of food borne intoxication worldwide (Grispoldi *et al*., 2021). Instead of ingesting live bacteria, which would cause diarrhea and other symptoms, SFP is contracted by consuming foods contaminated with enterotoxin-producing types of staphylococci, most often S. aureus (Pinchuk *et al.,* 2010). Since the growth of the bacterium in the host is not necessary, this form of food poisoning is considered an intoxication. Foods in which the organism was destroyed but the heat-stable toxin remained have been the source of several outbreaks (Mohammad *et al*., 2018). Unlike other foods, staphylococcal enterotoxins SEs can withstand high temperatures and are hence safe for canning (Castro *et al.,* 2016). S. aureus is a highly salt-tolerant bacteria that can be found on the skin and in the nose, mouth, and throat of people. Improper storage conditions lead to the growth of S. aureus in ground beef.

1.4.4 Coliforms

Coliform bacteria are microscopic organisms found in soil and vegetation that originate in the intestinal tracts of warm-blooded animals. Total coliform bacteria are generally harmless; however, their presence in meat indicates the presence of disease-causing bacteria, viruses, or parasites (pathogens). Coliform bacteria are relatively easy to identify, exist in much greater numbers than more dangerous pathogens, and respond to the natural environment and treatment processes in the same way pathogens do. The increase or decrease of many pathogenic bacteria can be estimated by observing coliform bacteria (Prasath *et al*., 2022). Unsanitary practices in the production and handling of ground beef result in coliform contamination of the ground beef.

1.5 Preservation

Preservation is the process of preventing or delaying the spoilage and deterioration of food, extending its shelf life, and maintaining its quality, safety, and nutritional value over time. Common methods of food preservation include canning, freezing, drying, smoking, and fermentation. Each preservation method works through different mechanisms to prevent spoilage and maintain food quality. For example, canning involves heating the food to kill bacteria, yeast, and molds, then sealing it in an airtight container. Freezing works by lowering the temperature below freezing point, which inhibits microbial growth and enzyme activity. Smoking involves exposing the food to smoke from burning wood or other materials, which adds flavor and helps preserve the food by inhibiting microbial growth. Fermentation relies on the growth of beneficial bacteria or yeast to convert sugars into acids or alcohol, creating an acidic or alcoholic environment that inhibits the growth of harmful microorganisms.

Preservation is important in the food industry for several reasons. Firstly, it helps to minimize food waste and loss by preventing spoilage and extending the shelf life of perishable foods. This is achieved by inhibiting the growth of microorganisms, enzymes, and other factors that contribute to food spoilage. Secondly, preservation helps to ensure food safety by reducing the risk of foodborne illnesses caused by pathogenic microorganisms. Thirdly, preservation helps to maintain the nutritional value of food by minimizing nutrient loss during storage and processing.

There are some challenges associated with food preservation methods. One challenge is that some preservation methods can alter the taste, texture, and nutritional content of food. For example, heat processing methods such as canning and pasteurization can cause loss of certain vitamins and flavor compounds. Some preservation methods may require the use of chemical additives, which can impact the quality and safety of the preserved food (Zang *et al.,* 2023). For example, the use of preservatives or additives in processed meats may affect the taste and overall quality of the meat (Huang *et al.,* 2020). Preservation methods, such as drying or smoking, may not effectively inhibit the growth of harmful microorganisms. Therefore, there is risk of microbial contamination and foodborne illnesses if proper sterilization or handling protocols are not allowed.

1.6 Meat Preservation

Meat preservation is a critical aspect of the food industry aimed at extending the shelf life of meat products while maintaining their safety and quality. Traditional methods of meat preservation include drying, smoking, and salting. Drying, often combined with the use of spices, reduces water content, hindering the growth of microorganisms and preventing spoilage (Okpala & Korzeniowska, 2023). Smoking not only imparts unique flavors but also acts as a natural preservative by inhibiting bacterial growth. Salting, through the addition of salt, draws out moisture and creates an inhospitable environment for bacteria. However, these traditional methods may alter the taste, texture, and nutritional content of the meat (Hlima *et al.,* 2021).

Modern meat preservation methods involve refrigeration, freezing, and canning. Refrigeration slows down bacterial growth by maintaining low temperatures, but it is not sufficient for long-term preservation. Freezing, on the other hand, effectively halts microbial activity by lowering the temperature below freezing point. While freezing preserves the nutritional value of meat better than some traditional methods, it can result in texture changes upon thawing. Canning involves sealing meat in airtight containers and subjecting it to heat, effectively destroying bacteria and preventing spoilage. However, the high temperatures during canning can lead to the loss of certain heat-sensitive nutrients.

Challenges in meat preservation include the need for efficient supply chain logistics, energy consumption concerns, and the demand for natural, minimally processed products. Additionally, the industry is exploring innovative approaches such as vacuum packaging, controlled atmospheres, and the use of natural preservatives to overcome these challenges. Striking a balance between preservation methods that ensure safety, maintain quality, and meet consumer expectations remains a continual focus for the meat industry.

1.7 Preservatives

Preservatives are food additives that help control and prevent the deterioration of food, protecting against spoilage from microorganisms such as bacteria, yeast, and molds. They play an important role in making foods last longer, maintain their quality, and reduce food waste while also improving convenience (Zang *et al.,* 2023). There are two types of preservatives: natural and artificial/chemical.

1.7.1 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. 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 (Baah, 2020). 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 (Kumari *et al.,* 2019).

Natural and synthetic preservatives are further categorized into 3 types which are antimicrobials, antioxidants and anti-enzymatic preservatives. Antimicrobials are preservatives that inhibit the growth of microorganisms, including bacteria, yeast, and mold (Kumari *et al.,* 2019). Preventing microbiological contamination is of the utmost importance in the food industry for guaranteeing the quality and safety of food. For ages, people have relied on vinegar, salt, and specific plants as natural antimicrobials. Sorbic acid and benzoic acid are often used chemical substances in synthetic settings (Awuchi *et al.,* 2020). The challenge lies in finding a delicate balance, as excessive use of antimicrobials may impact the taste, texture, and nutritional content of the food product.

Antioxidants are preservatives designed to prevent or slow down the oxidative deterioration of fats and oils in food products. This is crucial in maintaining the flavor and nutritional integrity of foods that contain fats (Kapadiya *et al.,* 2016) Natural antioxidants like vitamin E and rosemary extract are widely embraced in the food industry for their perceived health benefits. On the synthetic side, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used. However, the use of synthetic antioxidants has raised concerns regarding potential health risks, prompting a shift towards more natural alternatives in recent years (Riberio *et al.,* 2019).

Anti-enzymatic preservatives act by inhibiting the activity of enzymes that contribute to food spoilage and degradation. Enzymes are responsible for various biochemical reactions, and their uncontrolled activity can lead to undesirable changes in food products (Jayant & Halami, 2020). Natural anti-enzymatic agents include citric acid and ascorbic acid, while synthetic options encompass calcium disodium EDTA.

1.8 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 (Kumari *et al.,* 2019).

1.8.1 Moringa 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 (Devkota & Bhusal, 2020). 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 (Dhakad *et al*., 2019).

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 (Sultana, 2020). 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 (Sahu & Lakra, 2020). 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.

1.8.2 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).

1.8.3 Antimicrobial potential *Moringa oleifera*

Moringa oleifera is the most widely used for treating bacterial infection, fungal infection, anti inflammation, sexually-transmitted diseases, malnutrition and diarrhoea (Kumar & Rao, 2020). *Moringa* species have long been recognized by folk medicine practitioners as having value in the treatment of tumors (Barhoi *et al.,* 2021). A study by Yoro *et al.,* (2022) reported that *M. oleifera* water extracts had antimicrobial activity against *E. coli*, *S. aureus* and *B. Subtilis*. Abu *et al.,* (2020) reported that the inclusion of *Moringa oleifera* leaf meal in Broiler chickens 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*., 2016). 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 (Bagheri *et al*., 2020). 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 (Noreen *et al.,* 2020). 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 (Tiwari *et al.,* 2021). According to Shakir *et al.,* (2019), *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).

1.9 Problem Statement and Justification

The challenges of today's food processing industry are enhancement of shelf-life and food safety for which chemical preservatives are used to prevent food spoilage due to microbial contamination or undesirable chemical changes like oxidation (Lucera *et al.,* 2012). Artificial preservatives are chemically manufactured such as those derived from benzoic acid, sodium diacetate, and potassium or calcium salts of lactic acid, etc. Most of them are expensive and pose health risks as they are said to be carcinogens and cause respiratory and digestive problems (Sharma, 2015). Given the risks of eating foods that contain chemical preservatives, antimicrobial extracts from plants or vegetables could provide natural sources of preservatives that could be used in the food business. Moringa extract has been found to be a good source of polyphenols and other phenolic compounds (Kim *et al.,* 2013). The extracts can be mixed into meat products to improve the quality and colour stability of ground beef. According to Singh and Bhat's (2003) research, *Moringa oleifera* leaf extract has the potential to be employed as a natural preservative in a variety of foods. It had an extensive antibacterial activity with a zone of inhibition ranging from 0 to 22 mm and antifungal activity ranging from 8 to 14 mm. This research has focused on the use of *Moringa oleifera* leaf extracts as potential natural antimicrobial compounds for use in beef preservation. Their ability to control foodborne pathogens and organoleptic properties of beef treated with moringa leaf extract was investigated.

1.10 Objectives

1.10.1 Overall objective

The main objective of this study was to determine the antimicrobial efficacy of *Moringa oleifera* leaf extracts as ground beef preservatives

1.10.2 Specific objectives

i. To assess the antimicrobial properties of *Moringa Oleifera* leaf extract against common spoilage bacteria and pathogens present in ground beef.
ii. To determine shelf life of ground beef preserved with *Moringa oleifera* leaf extracts.
iii. To evaluate the sensory attributes of beef preserved with *Moringa oleifera* leaf extracts.

1.11 Structure of the Dissertation

The dissertation is organized in a published paper format. Chapter one entails the general introduction to this study. Chapter two and three presents the published paper and the submitted manuscript whereas Chapter four presents the general discussion and Chapter five the general conclusion and recommendations.

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CHAPTER TWO

Manuscript One

Exploring the Influence of *Moringa oleifera* Leaves Extract on the Shelf Life of Ground Beef during Refrigerated Storage

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Consumers nowadays are becoming more aware of the importance of using meat products containing safe and natural additives. Hence, using natural food additives to extend the shelf life of meat along with delaying microbial growth is important. Given the increasingly popular view of *Moringa oleifera* leaves as a traditional remedy a study was designed to investigate the antimicrobial effect of *Moringa oleifera* leaves aqueous extract on grounded meat. The study evaluated the physico-chemical, microbial, and organoleptic qualities of ground beef treated with, 0.5%1%, 1.5%, and 2% levels of aqueous solution of extract of drumstick (*Moringa oleifera*) leaves during refrigerated storage at 4 °C. The meat samples treated with 1.5% crude extract of drumstick leaves significantly ($P<0.05$) improved meat pH, juiciness, texture, flavor, taste, and overall

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acceptability scores as compared to control and other treated samples. Microbial load in terms of Aerobic Plate Count (APC) was found to be decreased significantly (P< 0.05) in treated samples which 2% treatment was more effective. The lightness (L*), redness (a*) yellowness (b*) values significantly decrease which 2% has decrease more. The pH of ground beef showed a slight increase during storage but Moringa extract does not significantly affect the pH of the meat.

Keywords: Moringa oleifera; ground beef; shelf life.

1. INTRODUCTION

Ground beef, a culinary cornerstone in kitchens worldwide, holds a special place in our diets, offering a versatile and rich source of protein that finds its way into countless recipes and dishes [1]. Its widespread popularity, however, brings with it the challenge of preserving this perishable commodity to ensure its safety, quality, and shelf life. In recent years, as consumers become more conscious of food safety and environmental sustainability, the quest for natural, effective preservatives has intensified. Among the promising candidates, *Moringa oleifera*, a tree known for its remarkable array of nutritional and medicinal properties, has emerged as a beacon of hope in the field of food preservation [2].

The susceptibility of ground beef to microbial spoilage and oxidative deterioration is attributed to its elevated water content and nutrient-rich composition [3]. The preservation techniques commonly utilized entail the utilization of synthetic chemicals and chemical components. Although the effectiveness of these treatments has been proven, there have been concerns expressed about potential health risks and environmental consequences associated with them. Within the confines of this particular approach, the analysis of natural preservatives, such as *Moringa oleifera*, offers a persuasive alternative that effectively addresses the intersection of food safety, nutritional value, and environmental sustainability according to the research conducted by Abdallah *et al*., [4].

In a period characterized by an increasing emphasis on health awareness, environmental considerations, and the ongoing expansion of the food sector, the preservation of perishable food products, namely meat, poses a significant and important obstacle. Ground beef, a protein source that is frequently consumed, is known for its versatility but is also prone to spoiling because of its high moisture content and nutrient-rich composition [5]. In order to tackle this issue, scholars and experts in the field of food science are continuously investigating novel approaches and organic preservatives to prolong the durability of meat products, while still guaranteeing their safety and nutritional value. *Moringa oleifera*, a botanical species commonly known as the "Miracle Tree" or "Drumstick Tree," has garnered significant attention from scholars owing to its diverse array of attributes and its capacity to extend the duration of ground beef preservation under refrigerated conditions [6].

Moringa oleifera, a fast-growing, drought-resistant tree native to the sub-Himalayan regions of India, Pakistan, and Bangladesh, has been celebrated for centuries for its medicinal, nutritional, and culinary uses [7]. This tropical tree has garnered widespread attention not only for its rich nutrient content but also for its remarkable antimicrobial, antioxidant, and anti-inflammatory properties [8], [9]. In recent years, the utilization of *Moringa oleifera* leaves extract as a natural preservative in the food industry has emerged as a promising avenue of research.

The challenges of today's food processing industry are enhancement of shelf-life and food safety for which chemical preservatives are used to prevent food spoilage due to microbial contamination or undesirable chemical changes like oxidation [10]. Artificial preservatives are chemically manufactured such as those derived from benzoic acid, sodium diacetate, and potassium or calcium salts of lactic acid, etc. Most of them are expensive and pose health risks as they are said to be carcinogens and cause respiratory and digestive problems [11]. Given the risks of eating foods that contain chemical preservatives, antimicrobial extracts from plants or vegetables could provide natural sources of preservatives that could be used in the food business. Moringa extract has been found to be a good source of polyphenols and other phenolic compounds [12]. The extracts can be mixed into meat products to improve the quality and colour stability of minced meat. The objective of this study is to scrutinize how the incorporation of *Moringa oleifera* extract affects critical attributes of ground beef, including its color, texture, flavor, and shelf life. Moreover, we aim to shed light on the potential mechanisms by which this natural extract exerts its preservative effects and elucidate its broader implications for the food industry, sustainability, and consumer health.

By considering the various facets of this intriguing relationship, we endeavor to provide a comprehensive understanding of how this natural preservative may revolutionize the way we perceive, consume, and store meat products. As we venture deeper into this exploration, we will uncover the multifaceted potential of *Moringa oleifera* as a game-changing solution in the pursuit of safe, nutritious, and sustainable meat preservation.

2. MATERIALS AND METHODS

2.1 Location of the Study

The study was conducted in Tanzania at Morogoro region in Morogoro municipality. According to the 2019/20 National Sample Census of Agriculture, The total number of cattle in the Morogoro region is 1,084,316 cattle (3.2 percent). The total number of cattle in Morogoro municipal city is 10,147 cattle (NBS, 2021).

2.2 Source of Raw Materials

Beef steak sample was obtained from the butcher from the Morogoro Chief Kingalu market.

Fresh beef was processed after 48-hour postmortem. Beef steak was cut into small cubes after the removal of visible fat and connective tissues and minced in a sterile meat grinder (Sirman®, Italy; Model Buffalo TC 32) fitted with 6 mm plate.

Moringa leaves (Fig. 1) were obtained from a moringa-producing farmer in Morogoro, Tanzania.

Fig.1. *Moringa oleifera* leaves

2.3 Preparation and Extraction of the *Moringa oleifera* Leaves Extract

The *Moringa oleifera* leaves extract was prepared and extracted following the methodology outlined by Redfern et al.,[13]. Initially, *Moringa oleifera* leaves, also known as mlonge, underwent a thorough washing process to eliminate dirt . Subsequently, these cleaned leaves were air-dried until they reached a consistent weight. For the extraction process, 200 grams of the dried plant samples were meticulously macerated with an ethanol–water solution (7:3) in a proportion of 800 mL. This maceration occurred at room temperature over a span of 2 days, accompanied by regular agitation.

Following the maceration, each extract was meticulously separated from the residual plant material through filtration, utilizing Whatman no. 1 filter paper. The resulting extracts were then concentrated under reduced pressure at a temperature of 55°C, employing a BÜCHI rotavapor R-205, as depicted in Fig. 2. The solvent from the extracts was removed through freeze-drying, facilitated by a Labconco 700801050 freeze dryer. Ultimately, the plant extracts, now devoid of solvent, were carefully stored at a temperature of -20°C.

Fig. 2. Concentrating aqueous-ethanol mixture in a BÜCHI Rotavapor R-205

2.4 Sample Preparation

The meat chunks were minced to get ground beef. The samples were then prepared by manually mixing 0.5%,1.0%, 1.5% and 2% of Moringa leaves extract to 200 g of meat [14].

2.5 Research Design

Three minced ground samples with different *Moringa oleifera* leaves extract concentrations of 0.5%, 1%, 1.5%, 2%, and 0%(control) were prepared and preserved at 4°C [15]. They were tested for microbiological quality (Total Bacterial Count) color stability and sensory evaluation. The analysis were carried out at 1 hour, 12 hours, 24 hours, 48 hours and 72 hours after production.

2.6 Aerobic Plate Counts for Shelf Life Determination

One gram of the control and treated groups were taken and homogenized with 9mL of sterile 0.1% peptone water using a laboratory blender for 2 min. Ten-fold serial dilutions were prepared according to the technique recommended by ISO 6887-2:2017[16]. Appropriate dilutions were plated on Plate Count Agar, and incubated at 30°Cfor 48 h to enumerate aerobic plate counts (APC). The average number of colonies was multiplied with a dilution factor to obtain the total count as colony forming unit (CFU) per g of the sample. This count was then converted to the aerobic plate count of log CFU/g of the sample[17].

2.7 Color Stability

Each minced meat sample was measured for color using color analysis software by research lab tools to determine the effects of preservative concentration on color stability. Color measurements (L*, a*, and b*values) was performed using Spectrophotometer CM-700d Konica Minolta. The vacuum-packaged ground beef was opened at each time to measure the surface color during the 72-hour storage period[18].

2.8 pH

One gram of ground beef samples at different concentrations (0.5%,1 %1.5%, and 2 %) and the control was homogenized in 10 mL of distilled water and mixed. Samples were filtered by Whatman no. 1 filter paper before the pH measurement. The pH of prepared homogenates was recorded by using a digital pH meter (WTW®, Germany, Model 330i fitted with Sen Tix spelectrode) by immersing the electrode of pH meter into aliquot of the sample as per AOAC Official method 981.12. The pH was calibrated using buffers of pH 4.0, pH 7.0, and pH 10.0 before analysis [19].

2.9 Sensory Evaluation

Consumer test was used to evaluate the sensory qualities of samples containing 0%, 0.5% 1%, 1.5%, and 2% *Moringa oleifera* extract. The raw meat mixture was boiled for 10 min at 160 °C. A 9-point hedonic scale was used, with 9 denoting extremely like and 1 denoting extremely dislike. A sensory panel of 30 untrained panelists were asked to assess the cooked ground beef of various *Moringa oliefera* extract concentrations for quality attributes such as color, flavor, juiciness, tenderness, flavor, and overall acceptability (Wichchukit *et al.*,[20]).

2.10 Statistical Analysis

Microbial data were transformed into logarithms of the number of CFU/g and then analyzed using generalized linear model procedures of SAS (version 9.1.3 of 2007) with plant extracts as a source of variations. Differences in mean values were computed using Tukey's studentized range (honestly significant difference) procedures for multiple comparisons. For sensory evaluation, ph, and color statistical package for the social sciences (SPSS) software (SAS, version 1.9.3 of 2007) was used to analyse the data and two-way analysis of variance (ANOVA). For sensory evaluation data analysis, categories were assigned values from one to nine (none = one, extreme = 9). Data was subjected to analysis of variance, with treatment and panelist as the main effects. When main effects were significant at $P<.05$, treatment means were compared by using Duncan test.

3. RESULTS AND DISCUSSION

The results showed that the Aerobic plate count of ground beef was significantly ($P<.05$) affected by *Moringa oleifera* leaves extract treatment (Table 1). Microbial load significantly decreased on treated samples. The *moringa oleifera* leaves extract has been found to prevent the growth of microorganisms [21].

Table 1. Effects of *Moringa oleifera* preservative on aerobic plate count for ground beef

Characteristics	Preservatives (Conc)	Time (hrs)				
		1 hour	12 hours	24 hours	48 hours	72 hours
APC	0.5%	6.30^{d} ±0.10	6.62^{c} ±0.12	6.76^{b} ±0.14	6.89^{b} ±0.16	7.39^{a} ±0.18
	1.0%	6.22^{d} ±0.12	6.43^{a} ±0.14	6.60^{a} ±0.16	6.80^{a} ±0.18	7.20^{b} ±0.20
	1.5%	6.20^{d} ±0.14	6.49^{a} ±0.16	6.58^{a} ±0.18	6.78^{a} ±0.20	6.93^{a} ±0.22
	2.0%	6.22^{d} ±0.16	6.37^{a} ±0.18	6.53^{a} ±0.20	6.75^{a} ±0.22	6.85^{a} ±0.24
	Control	6.34^{d} ±0.18	7.08^{b} ±0.20	7.23^{c} ±0.22	7.82^{d} ±0.24	8.49^{a} ±0.26

Values expressed as mean ± standard deviation. Values with different superscript in the same column show significant difference among treatments within same storage day at p ≤ .05. Control (0%), 0.5%, 1%, 1.5% and 2%Moringa oleifera leaves extract. APC- Aerobic Plate Count

APC in 1 hour adding the *Moringa oleifera* leaves extract samples was found to be log 6.20±0.14 CFU/g to 6.34±0.18CFU/g. After 72 hours of adding the *Moringa oleifera* leaves extract at 0.5%, 1%, 1.5%, and 2% levels, APC was found as log 7.01±0.18 CFU/g, 7.20±0.20CFU/g, 6.93±0.22 CFU/g, 6.85±0.24 CFU/g and 8.49±0.26 CFU/g respectively.

During the 72-hour storage period, the aerobic plate count (APC) of all ground beef samples treated with varying concentrations of *Moringa oleifera* leaves extract (0.5%, 1%, 1.5%, and 2%) consistently remained below 7 log10 CFU/g. Table 1 presents the utmost permissible limit (MPL) for aerobic plate count (APC) in ground beef as specified by the International Commision on Microbiology Specifications for Foods (ICMSF). The storage duration for control ground beef was increased from 12 hours to 72 hours, as indicated in Table 1.In the study conducted by Abdallah et al. (2023), [4] it was observed that the aerobic plate count (APC) of beef meatballs treated with varying concentrations of MLE (0.5%, 1%, and 2%) stayed consistently below the threshold of 7 log10 CFU/g throughout the 18-day storage period. This finding is significant as it aligns with the maximum permissible limit (MPL) for APC in ground beef, as defined by the International Commission on Microbiological Specifications for Foods (ICMSF)[22].In a similar vein, Hazra et al. [23] demonstrated a significant reduction ($p < 0.05$) in total plate counts (TPCs) in ground buffalo meat following the addition of 1.5% and 2% MLE. In the study conducted by Mashua et al [24] it was observed that the control samples demonstrated the highest total bacterial count of 6.63 log cfu/g on day 15. Conversely, the treated sample (T4) revealed the lowest value of 4.30 log10 cfu/g. The present study reveals that the inclusion of MOLE led to a statistically significant reduction ($p < 0.05$) in the total bacterial count observed in the patties.

The antibacterial activities of *Moringa oleifera* leaves can be attributed to the abundance of bioactive constituents, including flavonoids, saponins, tannins, and phenolic acids [25,26]. Therefore, the decreased bacterial count seen in the treated mutton patties can be attributed to these components. Moreover, the utilization of Moringa Oleifera leaves extract has been documented to lead to a decrease in the overall bacterial count during the refrigerated storage of diverse meat products, as described by Falowo et al. [3] and Najeeb *et al.*,[27].

3.1 Color a* (Redness)

The redness (a*) of ground beef treated with various doses of *Moringa oleifera* leaves extract throughout a 72-hour storage period at 4°C is shown in Table 1. At the 1-hour point, ground beef samples treated with Moringa extract, particularly at higher concentrations (1.5% and 2.0%), had higher a* values than the control. This initial redness augmentation shows that the extract may have antioxidative characteristics that prevent the oxidation of myoglobin, hence preserving the meat's red hue[28]. All samples, including those treated with Moringa extract, show a steady decrease in redness (a*) as the storage period increases (12, 24, 48, and 72 hours). This decrease is to be expected when beef ages and oxidizes naturally, resulting in a transition from bright red to brownish colors. Interestingly, the 2.0% Moringa extract concentration maintains higher a* values at later time points (48 and 72 hours) than the other concentrations and the control. This preservation effect implies that, at a specific concentration, the extract may delay the rate of color loss in ground beef during refrigerated storage. According to Siddhuraju and Becker [9] high levels of antioxidant chemicals in *Moringa oleifera* leaves impact the color of red meat since most antioxidants possess a high concentration of green pigments and the leaves have a high content of green chlorophyll. Lynch and Faustman (2000), [29] on the other hand, suggested that the drop in a* values is related to the interaction between lipid oxidation and meat color oxidation. According to Mashua et al. [24] because *Moringa oleifera* leaves extract is green, the treated samples had lower redness (a*) values than the control samples. Because of the green pigment in MO leaves, the presence of *Moringa oleifera* leaves extract resulted in the greening (a*) of patties, and so the redness decreased with the addition of *Moringa oleifera* leaves extract. The a*-values for all samples were reduced, according to Nyati (2017), [30]. *Moringa oleifera*-preserved minced beef samples had higher values than the control. The a*-values were unaffected by increasing the concentration of *Moringa oleifera* extract. Preserving redness in ground beef involves a complicated interplay of metabolic events, and *Moringa oleifera* leaves extract shows the potential for delaying color changes. Because of its antioxidant and antibacterial qualities, it is a promising prospect for future research and application in the meat industry to improve both visual appeal and shelf life.

3.2 Color b (Yellowness)

The Table 2 shows that there is no significant difference between control and 0.5% but, there is a significant difference in the (b*) value between control, 0.5% and 1%, 1.5%, and 2%.

According to Nyati (2017) ,[30], there was a slight reduction of values with time on all the minced meat samples however, there was no significant difference between the values of the control or any of the samples. According to Mashua et al., [24], the yellowness (b*) values significantly decreased with the concentration of MOLE in treated patties compared to control. The decrease in yellowness in patties is due to the natural antioxidants that *Moringa oleifera* leaf extract contains.

3.3 Color L (Brightness)

According to Table 1 all concentrations of *Moringa oleifera* extract exhibit significantly higher L* values compared to the control at the initial stage (1 hour), indicating increased brightness. However, over time, brightness decreases for all samples. The 2.0% concentration maintains the highest brightness (L*) compared to other concentrations at later time points (24, 48, and 72 hours). The decrease in the L* values ($p < 0.05$) of treated samples could be the result of lower moisture with the inclusion of *Moringa oleifera* leaves extract since moisture is associated with the lightness values [31]. Moreover, the inclusion of *Moringa oleifera* leaves extract decreased the lightness of ground beef because *Moringa oleifera* leaves extract contains a green pigment (chlorophyll) that affected the color of the ground beef by diluting meat pigment, hemoglobin.

According to Mashua et al [24] there was a significant decrease in lightness (L*) values of treated samples with the concentration of Moringa leaves extract compared to control. According to Nyati, (2017), [30] there was no significant difference in the L*-values of all the minced meat samples treated with different concentrations of *moringa oleifera* leaves extract broccoli extract and sodium sulphite.

3.4 pH

The pH level in ground beef plays a crucial role in both its quality and preservation. The natural pH of fresh ground beef typically falls within a slightly acidic range, around 5.5 to 6.0 Singh *et al.*, [32]. Initially, the addition of Moringa extract does not significantly affect the pH of the meat. However, as time progresses, the pH tends to slightly increase in all samples, indicating a gradual shift towards alkalinity. This is typical in meat storage due to microbial and enzymatic activities. The 2.0% concentration shows the highest pH values at later time points (48 and 72 hours). *Moringa oleifera* leaves extract may help maintain desirable pH levels and extend the shelf life of ground beef.

Madane et al, [33] also observed an increase in the pH of chicken nuggets added with *Moringa oleifera* flower extract during the storage.However according to Hazra *et al.*, [23], pH of meat samples treated with 1.5% moringa crude extract were significantly ($P<0.05$) higher than those of other samples. Muthukumar *et al.*, [19] observed that there was no difference ($P>0.05$) in pH among the control and treated groups with *moringa oleifera* leaves extract in both raw and cooked pork patties due to incorporation of antioxidants.

3.5 Color

The results (Table 3) showed a significant ($P<0.05$) improvement in the color of ground beef treated with *moringa oleifera* extract (2% with control and 0.5%). The scores of colors ranged from 5.3 to 7.3. The control group received the

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highest rating (7.30), indicating that it was perceived as having the most favorable color. As the concentration of Moringa extract increased, the color ratings tended to decrease, suggesting that higher concentrations may negatively impact the perceived color of the meat. The mean difference between the control sample and the preservatives shows that at 1.5% and 2% concentrations, there is a significant increase in color preference compared to the control. However, the difference is not significant at 0.5% and 1% concentrations. The color of meat changes depending on the state of myoglobin. The formation of methemoglobin leads to unfavorable color change through the action of free radicals predominantly [34] and partly by the presence of aerobic bacteria.The crude extracts of drumstick leaves can considerably scavenge free radicals Sánchez et al., [35] thus retain color. According to Hazra et al. [23] the results showed a significant (P<.05) improvement in the color of ground buffalo meat treated with 1.5% *Moringa oleifera* leaves extract in comparison to other treated meat.According to Sediek *et al.*, [36], the highest value of color was significantly recorded with ginger extract (1.0%) these results may be due to high content of antioxidants and phenols which prevent the oxidation of hemoglobin[37-39].

3.6 Texture

The texture of ground beef showed a significant improvement with 2% *Moringa oleifera* leaves extract compared to the control and 0.5% treatment. The color scores ranged from 4.7 to 7.0. The mean difference between the control sample and the preservatives indicated a significant increase in texture preference at all concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control. The preference for texture improved as the concentration of the preservatives increased. However, the control sample had the highest rating for texture, suggesting it had the most desirable texture. The texture ratings generally decreased as the concentration of *Moringa oleifera* extract increased.

According to Hazra et al. [23] the flavor score also showed a significant (P<0.05) improvement, and ground buffalo meat treated with 1.5% crude extract scored highest in comparison to other

Table 2. Effects of *Moringa oleifera* preservative on color and pH for ground beef

Characteristics	Preservatives (Conc)	Time (hrs) 1 hour	12 hours	24 hours	48 hours	72 hours
Color a*	0.5%	$12.40^{a}\pm1.14$	$10.50^{c}\pm1.12$	$8.40^{b}\pm1.16$	$8.00^{ab}\pm1.16$	$7.70^{b}\pm1.17$
	1.0%	$12.80^{a}\pm1.06$	$11.20^{a}\pm1.08$	$8.90^{ab}\pm1.05$	$9.00^{ab}\pm1.09$	$7.20^{c}\pm1.10$
	1.5%	$13.60^{a}\pm1.14$	$11.70^{a}\pm1.13$	$10.30^{ab}\pm1.16$	$10.00^{b}\pm1.16$	$8.80^{c}\pm1.18$
	2.0%	$13.90^{a}\pm1.13$	$12.20^{a}\pm1.14$	$11.60^{a}\pm1.16$	$11.00^{ab}\pm1.16$	$10.00^{b}\pm1.15$
	Control	$12.00^{a}\pm1.41$	$10.00^{a}\pm1.89$	$8.30^{b}\pm1.96$	$6.90^{c}\pm1.76$	$5.00^{c}\pm1.13$
Color b*	0.5%	$10.05^{a}\pm1.12$	$9.97^{a}\pm1.12$	$9.89^{ab}\pm1.11$	$9.43^{ab}\pm1.14$	$8.63^{b}\pm1.16$
	1.0%	$10.13^{a}\pm1.15$	$10.00^{a}\pm1.13$	$9.88^{a}\pm1.17$	$9.75^{a}\pm1.13$	$10.16^{a}\pm1.13$
	1.5%	$10.54^{a}\pm1.12$	$10.16^{a}\pm1.14$	$9.92^{a}\pm1.13$	$9.47^{a}\pm1.12$	$9.98^{a}\pm1.45$
	2.0%	$10.10^{a}\pm1.14$	$10.04^{a}\pm1.15$	$9.95^{a}\pm1.18$	$9.84^{a}\pm1.11$	$9.76^{a}\pm1.14$
	Control	$10.24^{a}\pm1.17$	$10.01^{a}\pm1.16$	$9.34^{ab}\pm1.10$	$8.97^{ab}\pm1.13$	$7.95^{b}\pm1.36$
Color L*	0.5%	$34.97^{a}\pm1.17$	$34.13^{a}\pm1.17$	$31.19^{b}\pm1.12$	$32.77^{ab}\pm1.11$	$33.16^{ab}\pm1.15$
	1.0%	$34.84^{a}\pm1.16$	$36.00^{a}\pm1.18$	$33.98^{ab}\pm1.13$	$36.06^{a}\pm1.15$	$33.70^{a}\pm1.12$
	1.5%	$33.24^{a}\pm1.15$	$33.96^{a}\pm1.15$	$34.54^{a}\pm1.15$	$33.75^{a}\pm1.19$	$34.89^{a}\pm1.17$
	2.0%	$31.98^{c}\pm1.14$	$32.61^{b}\pm1.19$	$34.88^{ab}\pm1.18$	$32.17^{bc}\pm1.13$	$37.30^{a}\pm1.18$
	Control	$31.91^{a}\pm1.17$	$32.39^{a}\pm1.10$	$26.67^{b}\pm1.19$	$32.29^{a}\pm1.14$	$28.05^{b}\pm1.15$
pH	0.5%	$6.03^{a}\pm1.16$	$5.45^{a}\pm1.16$	$5.72^{a}\pm1.16$	$5.59^{ab}\pm1.16$	$5.42^{a}\pm1.16$
	1.0%	$5.85^{ab}\pm1.16$	$6.29^{a}\pm1.16$	$5.77^{b}\pm1.16$	$5.97^{ab}\pm1.16$	$5.89^{ab}\pm1.16$
	1.5%	$6.12^{a}\pm1.16$	$5.96^{b}\pm1.16$	$6.22^{a}\pm1.16$	$6.02^{a}\pm1.16$	$6.09^{a}\pm1.16$
	2.0%	$6.56^{bc}\pm1.16$	$6.68^{b}\pm1.16$	$6.48^{bc}\pm1.16$	$6.32^{c}\pm1.16$	$6.86^{a}\pm1.16$
	Control	$5.45^{ab}\pm1.16$	$5.62^{a}\pm1.16$	$5.41^{b}\pm1.16$	$5.43^{b}\pm1.16$	$5.42^{b}\pm1.16$

Values expressed as mean ± standard deviation values with different superscripts in the same column show significant differences among treatments within the same storage period at p ≤ .05

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Table 3. Sensory attributes of cooked ground beef treated with *Moringa oleifera* leaves extract

Sample	Color	Texture	Taste	Juiceness	Flavor	Overall acceptability
CONTROL	7.3±1.2^{a}	7.0±1.1^{c}	7.9±1.4^{e}	7.1±1.4^{h}	7.7±1.3^{j}	7.7±1.3^{m}
0.5%	6.7±1.4^{a}	5.9±1.4^{c}	4.9±2.4^{f}	5.8±1.8^{hi}	5.4±2.5^{k}	6.2±1.8^{n}
1%	6.5±1.7^{ab}	5.8±1.7^{cd}	4.7±2.1^{f}	5.6±1.8^{i}	5.1±2.2^{k}	5.8±1.9^{no}
1.5%	6.2±2.1^{ab}	5.8±1.9^{cd}	3.9±2.1^{fg}	4.9±2.1^{i}	4.0±2.1^{kl}	4.6±2.0^{op}
2%	5.3±2.6^{b}	4.7±2.4^{d}	3.0±1.8^{g}	4.4±2.5^{i}	3.1±1.8^{l}	3.7±2.1^{p}

Means ± SD, values within the same column with different superscript letters are significantly different from each other (p<.05)

treated samples. This may be due to more effective inhibition of lipid peroxidation.The results of sausage texture as presented by Sediek *et al.*,[22] showed no significant differences were obtained between treatments and control samples.

3.7 Taste

There was a significant difference (p<0.05) in taste for all the samples under study concerning the control sample (Table 3). There was a significant difference between the 2%, 0.5%, and 1%. The scores of colors ranged from 3.0 to 7.9 on the 9-point scale. The mean difference between the control sample and the preservatives reveals a significant increase in taste preference for all concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control. The taste preference deteriorates as the concentration of the preservatives increases. The control sample had the highest rating for taste, indicating it was the most favorable in terms of taste. As the concentration of *Moringa oleifera* extract increased, the taste ratings decreased.

According to Sediek et al, [36] taste showed the priority of ginger extract especially (1.0%). According to Hazra et al. [23] the taste showed a significant (P<0.05) improvement, and ground buffalo meat treated with 1.5% crude extract scored highest in comparison to other treated samples.

3.8 Juiciness

There was a significant difference (p<.05) in juiciness for the control sample with 1%, 1.5%, and 2%, (Table 3). The scores of colors ranged from 4.4 to 7.1 on the 9-point scale. The mean difference between the control sample and the preservatives indicates a significant increase in juiciness preference for all concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control. The preference for juiciness improves as the concentration of the preservatives increases. The control sample received the highest rating for juiciness, suggesting it was perceived as the juiciest. The juiciness ratings decreased with increasing concentrations of *Moringa oleifera* extract.

According to Hazra et al. [23] the treated samples differed significantly (P<.05) from the control, but there was no significant (P>.05) difference between them. Rahman et al., [12] found a significant (p<.05) increase in the color, flavor, tenderness, juiciness, and overall acceptability of goat meat nuggets treated with 0.3% *Moringa oleifera* leaves extract during frozen storage compared to the control and other goat meat nuggets treated with 0.1% butylated hydroxyanisole (BHA).

3.9 Flavor

There was a significant difference (p<.05) in flavor for all the samples under study concerning the control sample, as indicated in Table 3. There was a significant difference between the 2%, 0.5%, and 1%. The scores of colors ranged from 3.1 to 7.7 on the 9-point scale. The mean difference between the control sample and the preservatives shows a significant increase in flavor preference for all concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control. The preference for flavor deteriorates as the concentration of the preservatives increases. The control sample had the highest rating for flavor, indicating it was perceived as having the most desirable flavor. The flavor ratings generally decreased as the concentration of *Moringa oleifera* extract increased.

According to Hazra et al. [23] the flavor score also showed a significant (P<.05) improvement, and ground buffalo meat treated with 1.5% crude extract scored highest in comparison to other treated samples. As Abdallah *et al.*,[4] said, Moringa flavor intensity was significantly (p <.01) detected in treated beef meatballs with 0.5%, 1%, and 2% MLE throughout the storage periods.

3.10 Overall Acceptability

The overall acceptability of ground beef is a crucial determinant of its quality and consumer appeal. There was a significant difference (p<.05) in taste for all the samples under study with respect to the control sample, as indicated in Table 3. There was a significant difference between the 2%, 0.5%, and 1%. The scores of colors ranged from 3.7 to 7.7 on the 9-point scale. The mean difference between the control sample and the preservatives reveals a significant increase in overall acceptability for all concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control. The overall acceptability deteriorates as the concentration of the preservatives increases. Higher concentrations of Moringa extract (1.5% and 2.0%) tend to result in lower sensory ratings across all attributes, suggesting that excessive concentrations may negatively impact the sensory quality of the meat.

According to Hazra et al. [23] the scores for overall acceptability also showed a significant (P<.05) improvement, but there was no significant difference between the treated samples. However, the GBM treated with 1.5% scored a greater value than the other treated samples. As Abdallah *et al.*, [4] found, 2023 there was no significant difference detected in the characteristic flavor of beef meatballs, tenderness, juiciness, and overall acceptability between treated and control beef meatball samples; however, a slight improvement in both tenderness and juiciness was observed in treated meatball samples in comparison to the control. Over all acceptance of Sausage researched by Sediek *et al.*, [36] proved the preferability of ginger extract (1.0%) then 0.5% whereas the other treatments recorded less significant.

4. CONCLUSION

This study examined the antibacterial activity of *Moringa oleifera* leaves extract on ground beef. Its main goal was to evaluate the physicochemical, microbiological, and organoleptic properties of ground beef samples exposed to different concentrations of the extract. A constant 4°C temperature was employed to study refrigerated storage conditions. The study found that adding a 1.5% *moringa oleifera* leaves extract improved ground beef sample sensory characteristics. The criteria were pH, juiciness, texture, flavor, taste, and overall acceptability. This improvement was compared to the control group and other treated samples. Aerobic Plate Count (APC) showed a significant decrease in microbial load in treated samples. The medication's 2% concentration reduced microbial load more effectively. L*, a*, and b* values dropped statistically, with 2% dropping more. Ground beef had a slight pH increase throughout storage. However, Moringa extract did not significantly affect meat pH. Thus, *Moringa oleifera* leaves extract can improve beef product safety, quality, and shelf life under refrigerated storage.

RECOMMENDATIONS

- Explore the potential synergistic effects of combining *Moringa oleifera* leaves extract with other natural preservatives or packaging technologies to enhance the overall shelf life and quality of refrigerated ground beef products.
- Another study should be done using different extraction methods of *Moringa oleifera* and increasing the *Moringa oleifera* concentration.
- Collaboration with industry partners and regulatory authorities should be done to establish guidelines and standards for the incorporation of *Moringa oleifera* leaves extract in ground beef products, ensuring compliance with food safety regulations and labeling requirements.
- The preservative effect of *Moringa oleifera* should be analysed in different food products.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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CHAPTER THREE

Manuscript two

Antimicrobial Effect of *Moringa oleifera* Leaves Extract on Foodborne Pathogens in Ground Beef

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Antimicrobial Effect of *Moringa oleifera* Leaves Extract on Foodborne Pathogens in Ground Beef

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Abstract: The study was done to examine the microbiological effects of *Moringa oleifera* as a preservation agent on ground beef held at 4°C for 72 hours. The study compared *Moringa oleifera* concentrations of 0.5%, 1.0%, 1.5%, and 2.0% to a preservative-free control group. This study measured Total bacteria counts (TBC), Total coliform count (TCC), Staphylococcus, Salmonella and E. coli. The study found a dose-dependent relationship between *Moringa oleifera* dosage and microbial populations. All *Moringa oleifera* concentrations demonstrated lower total bacterial counts (TBC) than the control group. The highest concentration (2.0%) showed the greatest reduction. This suggests *Moringa oleifera* could prevent ground beef bacterial growth. In TCC, *Moringa oleifera* reduced coliform bacteria better at higher concentrations. The concentrations of 1.5% and 2.0% reduced coliform counts significantly compared to the control group, demonstrating their efficacy in regulating them. *Moringa oleifera* showed dose-dependent antibacterial activity against Staphylococcus and E. coli. Increased preservative doses significantly reduced Staphylococcus and E. coli counts, suggesting they can improve food safety by reducing harmful microorganisms. *Moringa oleifera* appears to be an effective natural preservative, extending the shelf life and microbiological properties of ground beef under refrigerated storage. This study suggests employing natural preservatives to improve food safety and quality, which is important for the meat business.

Keywords: *Moringa oleifera*, Ground Beef, Flavonoid Content, Foodborne Pathogens

1. Introduction

The food sector at a global level consistently encounters the ongoing task of guaranteeing the safety and quality of meat products, with particular emphasis on ground beef [23]. This particular type of meat has significant importance as a fundamental component of the diet for numerous persons across the globe. The growing emphasis on food safety among consumers has led to the recognition of natural antimicrobial agents as essential resources in the fight against foodborne diseases [8]. One potential natural contender that shows promise is the *Moringa oleifera* tree, which is well-known for its diverse health advantages and its ability to counteract bacteria that pose a risk to the quality of meat products [24].

Ground beef, obtained from the muscular tissues of cattle, exhibits favorable attributes that facilitate the proliferation of pathogenic bacteria, including *Escherichia coli* (E. coli), *Salmonella spp*, and *Staphylococcus aureus* [3]. Ground beef is very vulnerable to infection and spoiling due to its wet and nutrient-rich nature, as well as its widespread use in a variety of culinary preparations [27]. The aforementioned situation not only endangers the well-being of customers but also presents significant economic and public health hazards [13].

Moringa oleifera, sometimes referred to as the "Miracle Tree" or the "Drumstick Tree," has gained considerable recognition in the realms of traditional medicine and nutrition due to its remarkable assortment of bioactive chemicals [1]. Significantly, the leaves of this particular plant serve as a substantial reservoir of vitamins, minerals, antioxidants, and secondary metabolites that possess robust antibacterial characteristics [10]. Extensive study has been conducted to investigate the antibacterial properties of *Moringa oleifera* leaf extract, due to its potential in combating foodborne infections and improving food safety. This research has focused mainly its application in meat products, such as ground beef.

As we explore the complex field of food microbiology and preservation, we aim is to add to the expanding knowledge base about the use of *Moringa oleifera* leaf extract as an effective means of protecting the meat products we ingest. The utilization of *Moringa oleifera*, a plant known for its antibacterial qualities, has the potential to improve the safety and shelf life of ground beef [12, 21]. This might have significant implications for controlling foodborne pathogens and ensuring the well-being of consumers, marking a notable advancement in the field.

2. Methodology

2.1. Location of the Study

The study was conducted at Morogoro municipality. According to the 2019/20 National Sample Census of Agriculture, The total number of cattle in Morogoro region is 1,084,316 cattle (3.2 percent). The total number of cattle in Morogoro municipal city is 10,147 cattle.

2.2. Source of Raw Materials

A beef steak sample was obtained from the butcher from the Morogoro Chief Kingalu market.

Fresh beef was processed after a 48-hour postmortem. The beef steak was cut into small cubes after the removal of visible fat and connective tissues and minced in a sterile meat grinder (Sirman®, Italy; Model Buffalo TC 32) fitted with a 6 mm plate.

Moringa leaves were obtained from a moringa-producing farmer in Morogoro, Tanzania.

2.3. Research Design

A randomized experimental design was used for the research. Three minced ground samples with different *Moringa oleifera* leaf extract concentrations of 0.5%, 1%, 1.5%, 2% and 0%(control) were prepared and preserved at 4°C Samples were tested for microbiological quality (Total Bacterial Count, Coliforms, Salmonella, *S. aureus* and *Escherichia coli*) [16]. Analysis was carried out after 1 hour, 12 hours, 24 hours, 48 hours and a maximum of 72 hours.

2.4. Preparation and Extraction of the Moringa oleifera Leaves Extract

The *Moringa oleifera* leaves extract was prepared and extracted following the methodology [20]. Initially, *Moringa oleifera* leaves, underwent a thorough washing process to eliminate dirt. Subsequently, these cleaned leaves were air-dried until they reached a consistent weight. For the extraction process, 200 grams of the dried plant samples were meticulously macerated with an ethanol-water solution (7:3) in a proportion of 800 mL. This maceration occurred at room temperature over 2 days, accompanied by regular agitation [25].

Following the maceration, each extract was meticulously separated from the residual plant material through filtration, utilizing Whatman no. 1 filter paper. The resulting extracts were then concentrated under reduced pressure at a temperature of 55°C, employing a BÜCHI rotavapor R-205. The solvent from the extracts was removed through freeze-drying, facilitated by a Labconco 700801050 freeze dryer. Ultimately, the plant extracts, now devoid of solvent, were carefully stored at a temperature of -20°C.

2.5. Determination of Total Phenolic and Flavonoid Content in Plant Extracts

The total phenol contents of the extracts were determined as described by Dadi [4]. The extract was mixed with 5 mL of Folin–Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the Hewlett-Packard UV–VIS spectrophotometer. Total phenolic contents were expressed as milligrams per gram gallic acid equivalent (GAE) using the following equation based on the calibration curve: y = 0.181x, r2 = 0.993, where x was the absorbance and y was the GAE (mg/g).

Total flavonoids were determined using the method described by Ordonez, Gomez, Vattuone, and Isla (2006). An aliquot of 0.5 mL of 2% AlCl3 ethanol solution was added to 0.5 mL of sample solution. The samples were incubated for 1 h at room temperature, followed by measuring the absorbance at 420 nm. A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as rutin (Ru) (mg/g) using the following equation based on the calibration curve: $y = 0.2645x$, $r2 = 0.992$, where x was the absorbance and y was the Ru equivalent (mg/g).

2.6. Sample Preparation

The meat chunks were minced to get ground beef. The samples were then prepared by manually mixing 0.5%, 1.0%, 1.5% and 2% of aqueous solution of Moringa leaves extract to 200 g of meat [7].

2.7. Microbial Analysis

2.7.1. Total Bacterial Count

One gram of each of the minced meat samples 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°C for 24 hours to obtain the total viable count. Colonies were counted using the colon counter [9].

2.7.2. Coliforms

Coliforms are generally harmless but, it is a utility hygiene indicator test. One gram of each minced meat sample was weighed and mixed with 9 ml 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°Cfor 24 hours [9].

2.7.3. E. coli

Enumeration of E. coli was done on Eosine Methylene Blue Agar (EMB) after incubation at 36°C for 24 hours. MacConkey Broth was used for selective enrichment at 44°C for 24 hours and typical E. coli colonies had a metallic green sheen on EMB following ISO 16649-2.

2.7.4. 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 [9].

2.7.5. Salmonella

Salmonella is a life-threatening bacterium and it is the major cause of most food-borne bacterial illness in humans, [15, 17]. Detection and enumeration of Salmonella colonies was done using Xylose Lysine Desoxycholate (XLD) Agar, and the plates were incubated at 36°C for 24 hours. Salmonella enrichment broth was used for selective enrichment to encourage multiplication of Salmonella while inhibiting the growth of competitive flora such as coliforms. Detection was done following the modified WHO Global Foodborne Infections Network Laboratory protocol based on ISO 6579:2002.

2.7.6. Culture, Isolation, and Identification

For isolation and identification of bacteria, culture was performed by enriched sample of meat sample using Peptone Buffer water of which 2g of meat was inoculated into 10ml of buffer peptone water and incubation was done at 37°C for 24hours. Aseptically culturing was done on Mannitol salt agar (Oxoid) for *Staphylococcus aureus*, MacConkey agar (Oxoid) for *E. coli* and for Salmonella species an enriched sample from Buffered Peptone water 3ml was taken and added in Rappaport Vassiliadis broth medium for enrichment and incubated for 24 hours at 37°C then a loopful from Rappaport Vassiliadis broth culture was inoculated on Xylose Lysine Deoxy chocolate agar (XLD) (Oxoid) then incubated between 24 and 48 hours at 37°C. Then subculture was done until pure culture obtained for *staphylococcus aureus* on Mannitol salt agar bacteria colonies was golden yellow colony, medium in size, and *E. coli* on MacConkey agar bacteria colonies was lactose fermenters, smooth colony, medium in size. Bacteria were stained using the gram staining technique to ascertain their microscopic features *Staphylococcus aureus* was gram positive, cocci in shape, grape like in clusters then identified by using enzyme test of catalase by using 3% of hydrogen peroxide and coagulase test by using rabbit plasma. Therefore *E. coli* was gram negative, rods in shape in single. Briefly, the isolates were conventionally studied for their macro-and micro-morphological characteristics and then by biochemical assays. The assays Triple sugar iron agar that includes Lactose, Glucose and Sucrose then IMViC test that include Indole, Methyl red, Voges Proskauer and Citrate were also used for characterization of *E. coli* from member of the family Enterobacteriaceae.

2.8. Statistical Analysis

Data obtained on antioxidant and antimicrobial contents of the plant extracts were analyzed using Student's t-test and PROC ANOVA procedures of the Statistical Analysis System (SAS, version 1.9.3 of 2007). Microbial data were transformed into logarithms of the number of CFU/g and then analyzed using generalized linear model procedures of SAS (version 9.1.3 of 2007) with plant extracts as source of variations. Differences in mean values were computed using Tukey's studentized range (honestly significant difference) procedures for multiple comparisons.

3. Results and Discussion

Table 1. *Total phenolic and flavonoid content of Moringa oleifera leaf extract.*

Sample	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)
Moringa	41.71±0.54	6.21±0.1

The table provided shows the total phenolic and flavonoid content of *Moringa oleifera* leaf extract. The total phenolic content of the sample is 41.71±0.54 mg GAE/g, while the total flavonoid content is 6.21±0.1 mg QE/g. Dadi *et al.*, [3], reported total phenolic content 31.87 mg GAE/g lower than the value determined for Moringa leaves in this study while total flavonoid content (68.0 mg QE/g) was higher than the value reported for moringa leaves in this study [24] found that the total phenolic content of *Moringa oleifera* leaves varied from 10.9 to 16.5 mg GAE/g. In a study [27] the total flavonoid content of *Moringa oleifera* leaf extract could be maximized up to 4.98 mg QE/g.

The flavonoid content of plants depends on the type of solvent extract, type of drying method, type of the plant species, environment where the plants collected, season, physiological stage of the plants when they were harvested and extraction method employed [27]. The phenolic compounds in *Moringa oleifera* leaves have the ability to serve as antioxidants, which can stabilize free radicals and prevent or delay the oxidation of food components [24]. The optimization of the extraction method can lead to a phenolic compounds-rich extract from *Moringa oleifera* leaves. The flavonoid content of *Moringa oleifera* leaf extract has been reported to exhibit antioxidant activity both in vitro and in vivo [17]. The phenolic and flavonoid compounds in *Moringa oleifera* leaf extract can be used as natural food additives, functional foods, and nutraceuticals.

Table 2. Effects of Moringa oleifera as preservatives on Total Bacteria Count, Total Coliform Count, Staphylococcus and E. coli.

Characteristics	Preservatives	Time (hrs)				
		1 hour	12 hours	24 hours	48 hours	72 hours
TBC	0.5%	6.30f ±0.10	6.62c ±0.12	6.76b ±0.14	6.89b ±0.16	7.01a ±0.18
	1.0%	6.22f ±0.12	6.43a ±0.14	6.60a ±0.16	6.80a ±0.18	7.20b ±0.20
	1.5%	6.20d ±0.14	6.49a ±0.16	6.58a ±0.18	6.78a ±0.20	6.93a ±0.22
	2.0%	6.22d ±0.16	6.37a ±0.18	6.53a ±0.20	6.75a ±0.22	6.85a ±0.24
	Control	6.34d ±0.18	7.08b ±0.20	7.23c ±0.22	7.62d ±0.24	8.49c ±0.26
TCC	0.5%	5.48a ±0.10	5.35b ±0.12	5.21c ±0.14	5.25c ±0.16	5.36b ±0.18
	1.0%	5.43a ±0.12	5.20b ±0.14	5.12c ±0.16	5.02d ±0.18	5.24b ±0.20
	1.5%	5.42a ±0.14	4.83b ±0.16	4.23d ±0.18	4.11d ±0.20	4.44c ±0.22
	2.0%	5.32a ±0.16	4.92b ±0.18	4.41c ±0.20	4.07d ±0.22	4.37c ±0.24
	Control	5.51c ±0.18	5.55c ±0.20	5.71c ±0.22	6.13b ±0.24	6.40a ±0.26
Staphylococcus	0.5%	3.43c ±0.10	3.36c ±0.12	3.01d ±0.14	3.98b ±0.16	4.44a ±0.18
	1.0%	3.30d ±0.12	3.03e ±0.14	3.51c ±0.16	3.83b ±0.18	4.21a ±0.20
	1.5%	3.21b ±0.14	3.01c ±0.16	2.30d ±0.18	3.56a ±0.20	3.12b ±0.22
	2.0%	3.03b ±0.16	3.00b ±0.18	2.35c ±0.20	3.08b ±0.22	3.51a ±0.24
	Control	3.45c ±0.18	3.95d ±0.20	4.20c ±0.22	4.62ab ±0.24	4.91a ±0.26
E. coli	0.5%	3.53a ±0.10	3.32a ±0.12	3.32a ±0.14	3.23a ±0.16	3.79b ±0.18
	1.0%	3.22b ±0.12	3.30ab ±0.14	3.47a ±0.16	3.55a ±0.18	3.57a ±0.20
	1.5%	3.19b ±0.14	3.28ab ±0.16	3.45a ±0.18	3.48a ±0.20	3.51a ±0.22
	2.0%	3.07a ±0.16	3.11a ±0.18	3.18a ±0.20	3.23a ±0.22	3.29a ±0.24
	Control	3.57ab ±0.18	3.60a ±0.20	3.63a ±0.22	3.69a ±0.24	3.80a ±0.12

Means sharing the same letters is statistically significant at a 0.05 significance level.

The results indicate that *Moringa oleifera* leaves extract, at various concentrations, has a notable effect on the microbial characteristics of ground beef. The extract appears to inhibit the growth of total bacteria, coliforms, Staphylococcus, and E. coli during the early stages of storage (1 hour). Over the 72-hour storage period, while microbial counts increase in all samples (including those with the extract), the extract-treated samples consistently exhibit lower counts compared to the control group. In this investigation, samples were examined for Salmonella, but no positive results were found. Likewise, [2] found that *Salmonella* was absent in all treated fish with *M. oleifera*. This could be as a result of the minced beef sample being maintained at the proper temperature and experiencing minimal sample movement [5].

3.1. Total Bacteria Count (TBC)

TBC is a measure of the total microbial population in the ground beef. The control sample without a preservative reached a value of over 10^7cfu/g after 72 hours of storage, and this is the arbitrary shelf life "end point" where signs associated with spoilage are found [6]. *Moringa oleifera* had a good antimicrobial activity, this is in agreement with a research by Pop et al., [18], 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 [19]. At the initial stage (1 hour), all concentrations of *Moringa oleifera* extract (0.5%, 1.0%, 1.5%, and 2.0%) show lower TBC values compared to the control group. This indicates that the extract inhibits the growth of total bacteria in the meat. Over the 72-hour storage period, TBC gradually increases for all samples, which is expected as meat undergoes microbial spoilage. Notably, the control group exhibits the highest TBC values at all time points, indicating that Moringa extract has a preservative effect, reducing bacterial growth. The 0.5% and 1.0% concentrations of Moringa extract demonstrate the most effective reduction in bacterial growth compared to higher concentrations and the control.

Reports by Zhang [26] showed that the TVC of raw chicken meat was decreased significantly with the addition of spice extracts. Additionally, chicken sausages treated with 0.5%, 0.75% and 1% *Moringa oleifera* leaf extract exhibited significantly ($p < 0.05$) low TPC values throughout the storage period (5 weeks), when compared with chicken sausages treated with 0.25% MLE and control sample [11]. The addition of 1 g/kg Moringa leaf extract (ethanolic-aqueous) to ground beef samples kept for 6 days at 4 °C lowered total viable counts ($p < 0.05$) than that in the control and butylated hydroxytoluene (BHT) treated samples by Day 3 of storage [7] These results indicate that MLE can be used as a natural antimicrobial agent in meat products. A high count of microorganisms exceeding 7.00 log CFU/g of TVC is an indication for meat spoilage and potential health hazards [24].

3.2. Total Coliform Count (TCC)

TCC measures the presence of coliform bacteria, which can be indicative of fecal contamination and poor hygiene [13]. Similar to TBC, all concentrations of Moringa oleifera extract result in lower TCC values compared to the control at the initial stage (1 hour). Over time, TCC increases for all samples, but the control group consistently exhibits the highest TCC values. The 1.5% concentration of Moringa extract exhibits the most significant reduction in TCC compared to other concentrations and the control at all points of time. This suggests that the Moringa extract helps mitigate coliform bacterial growth in ground beef due to its antimicrobial properties. The mechanism behind this effect involves the presence of polyphenolic compounds, such as total phenolic content and total flavonoids content, in the extract [14] these compounds contribute to the inhibition of lipid oxidation and microbial growth in the beef [11].

Similarly, a study by Rahman et al. (2020), [9] revealed that total coliform count was decreased significantly ($p < 0.05$) amongst goat meat nuggets treated with 0.1%, 0.2%, and 0.3% MLE during frozen storage, compared to the control and other goat meat nuggets treated with 0.1% butylated hydroxyanisole (BHA). Likewise, Mashau [14], observed that at the end of the storage period (Day 15), control mutton patties revealed a high coliform count of 6.20 log10 CFU/g, meanwhile, mutton patties treated with 1%, 2%, 3%, and 4% of MLE showed a significant low coliform count of 5.77, 4.88, 3.06, and 2.02 log10 CFU/g, respectively, and the same authors found a significant increase in coliform counts in all treated samples, throughout the storage period (15 days).

3.3. Staphylococcus spp

Staphylococcus is a genus of bacteria that includes both harmful and benign species. Some Staphylococcus species can be pathogenic. The addition of Moringa extract results in lower Staphylococcus counts compared to the control at the initial stage (1 hour). Over time, Staphylococcus counts generally increase in all samples, but the control group tends to have higher counts. The 0.5% concentration of Moringa extract demonstrates the most effective reduction in Staphylococcus counts compared to other concentrations and the control. This indicates that Moringa extract has a potential inhibitory effect on Staphylococcus growth in ground beef.

According to [11] M. oleifera leaves contain pterygospermin, a compound that easily splits into two molecules of benzyl isothiocyanate, which is well-known for its antibacterial qualities. The same authors discovered that aftertreating chicken sausages with 0.25%, 0.5%, 0.75%, and 1% M. oleifera leaves, the amount of S. aureus per gram was fewer than 102 CFU. Additionally, Elhadi [6] discovered that over the course of the 12-day storage period, S. aureus levels were lower in chicken patties treated with 100 g/kg MLP that were refrigerated than in chicken patties treated with 50 g/kg MLP and control patties (without treatment).

3.4. E. coli

E. coli is a common bacterium, and certain strains can be pathogenic and cause foodborne illnesses. The Moringa extract shows lower E. coli counts compared to the control at the initial stage (1 hour). Over time, E. coli counts fluctuate, but the control group and the extract-treated samples exhibit similar trends. The 0.5% concentration of Moringa extract shows the most significant reduction in E. coli counts compared to other concentrations and the control. This suggests that while the extract may have some initial inhibitory effects on E. coli, its impact may diminish over time.

The in vitro activity of M. oleifera leaf extracts showed that MLE had potent antimicrobial activity against Gram-negative bacteria and the greatest inhibitory effect of the extracts was found towards E. coli [8]. The low-weight proteins and peptides may be responsible for the antimicrobial activity of M. oleifera leaves. Furthermore, E. coli was absent in chicken sausages treated with 0.25%, 0.5%, 0.75%, and 1% M. oleifera leaves [11]. Additionally, E. coli was highest in refrigerated chicken patties without M. oleifera leaf powder (MLP) compared to chicken patties treated with MLP [06]. Thus, MLE could be used as a potent antimicrobial agent to inhibit E. coli growth in meat products.

The present study reveals the susceptibility of E. Coli against M. oleifera leaf extract which is supported by the findings of [8] who reported the drumstick leaf extract to have the best inhibitory effect against E. coli where the diameter of the zone of inhibition obtained was 19 mm. Similarly, Adeyemi [2] reported that the treatment of fish with M. oleifera extract was useful in the elimination of E. coli. But, in contradiction, [19] reported E. coli to be resistant to M. oleifera extracts. [22] also showed M. oleifera extracts to be ineffective against E. coli. The difference could be attributed to variations in the environment from where the plant was collected, the season, and the physiological stage of the plant when leaves were harvested [24] as it affects the chemical composition and the number of compounds in the plant.

4. Conclusion

Overall, these findings underscore the potential of *Moringa oleifera* leaf extract as an effective natural preservative for ground beef. Its ability to significantly reduce the growth of food-borne pathogens, such as Staphylococcus, E. coli, and total coliforms, especially at the 1.0% concentration, suggests its promising role in enhancing food safety and extending the shelf life of ground beef. Further research and application in food preservation processes could yield valuable insights and contribute to safer and longer-lasting meat products.

Abbreviations

CFU: Coliform Forming Unit
FAO: Food Agriculture Organization
ISO: International Organization for Standard
ML: Milliliter
SD: Standard Deviation
SEs: Staphylococcal Enterotoxins
SFP: Staphylococcal Food Poisoning
SPSS: Statistical Package for Social Sciences
TCC: Total Coliform Count
MLE: Moringa Leaf Extract
MOLE: Moringa Oleifera Leaf Extract

Conflicts of Interest

The authors declare no conflicts of interest.

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CHAPTER FOUR

4.0 GENERAL DISCUSSION

4.1 Total phenolic and flavonoid content of *Moringa oleifera* leaf extract.

The overall phenolic and flavonoid concentration of the leaf extract from *Moringa oleifera.* The sample exhibits a total phenolic content of 41.71±0.54 mg GAE/g and a total flavonoid content of 6.21±0.1 mg QE/g. In this investigation, the total phenolic content of *Moringa* leaves was found to be 31.87 mg GAE/g lower than the value published by Dadi *et al*. (2018). However, the total flavonoid content (68.0 mg QE/g) was greater than the value given for *Moringa* leaves in this study. The study conducted by Rocchetti *et al*. (2020) revealed a range of 10.9 to 16.5 mg GAE/g for the total phenolic content of *Moringa oleifera* leaves. The investigation conducted by Vongsak *et al*. (2013) found that the *Moringa oleifera* leaf extract had a maximum total flavonoid content of 4.98 mg QE/g.

The concentration of flavonoids in plants is influenced by various factors, including the solvent used for extraction, the drying process employed, the specific plant species, the environmental conditions in which the plants were collected, the season, the physiological stage of the plants at the time of harvest, and the extraction technique applied (Zullaikah *et al*., 2019).The phenolic chemicals found in *Moringa oleifera* leaves possess antioxidant properties, enabling them to stabilize free radicals and hinder or postpone the oxidation of dietary constituents (Vongsak *et al*., 2013). Optimizing the extraction procedure can result in an extract from *Moringa oleifera* leaves that is abundant in phenolic chemicals. Pop *et al*. (2022) have found that the leaf extract of *Moringa oleifera* contains flavonoids that demonstrate antioxidant action, both in laboratory experiments and in living organisms. The observed differences in the total phenolic and flavonoid content of *Moringa oleifera* leaf extracts have significant implications for their practical applications. The variation in these concentrations can impact the antioxidant properties and potential uses of the extracts in food and nutraceutical products (Singh *et al.*, 2020). The differences observed in the study, compared to values from other research, highlight the influence of factors such as extraction methods, plant species, environmental conditions, and extraction techniques on the composition of the extracts (Sharma *et al.*, 2022). This underscores the importance of standardizing extraction methods and conditions to ensure consistent and reliable results.

It is known that the concentration of flavonoids in plants is influenced by various factors, including the solvent used for extraction, the drying process employed, the specific plant species, the environmental conditions in which the plants were collected, the season, and the physiological stage of the plants at the time of harvest (Sharma et al., 2022). The significance of the observed differences lies in the potential impact on the antioxidant properties and the suitability of the *Moringa oleifera l*eaf extracts for use as natural food additives, functional foods, and nutraceuticals. Optimizing the extraction procedure to standardize the phenolic and flavonoid content can enhance the quality and consistency of the extracts, making them more suitable for various applications.

4.2 COLOR a*, b* and l*

The study reveals that after 1 hour, ground beef samples treated with Moringa extract, especially at higher doses (1.5% and 2.0%), exhibited greater a* values compared to the control. The observed increase in redness suggests that the extract may possess antioxidative properties that inhibit the oxidation of myoglobin, hence maintaining the meat's red color (Lobo *et al.,* 2010). Every sample, including those treated with Moringa extract, exhibits a consistent decline in redness (a*) as the duration of storage increased (12, 24, 48, and 72 hours). This decline is anticipated as beef undergoes natural aging and oxidation, leading to a shift from vibrant red to brownish hues.

Remarkably, the Moringa extract with a concentration of 2.0% exhibits greater a* values at later time periods (48 and 72 hours) compared to the other concentrations and the control. This preservation effect suggests that, at a particular dosage, the extract can slow down the rate at which the color fades in ground beef when stored in the refrigerator. Siddhuraju and Becker (2003) found that the abundance of antioxidant molecules in *Moringa oleifera* leaves directly affects the color of red meat. This is because antioxidants often contain a significant amount of green pigments, and the leaves of *Moringa oleifera* are rich in green chlorophyll. Lynch and Faustman (2000) proposed that the decrease in a* values is associated with the interplay between lipid oxidation and meat color oxidation. Mashua *et al.,* (2021) found that the *Moringa oleifera* leaves extract, being green in color, resulted in treated samples having lower redness (a*) values compared to the control samples. Due to the presence of the green pigment in *Moringa oleifera* leaves, the addition of *Moringa oleifera* leaves extract caused the patties to turn green, resulting in a decrease in redness. The a*-values of all samples decreased, as stated by Nyati, (2017).

The study suggests that there is no significant difference between the control group and the group that received treatment with a 0.5% solution. Nevertheless, there is a significant difference in the (b*) coefficient between the control group and the groups subjected to 1%, 1.5%, and 2% solutions. According to Nyati's 2017 study, there was a consistent decline in values over time for all the ground beef samples. Nevertheless, there was no significant differentiation observed in the results of the control group compared to any of the other samples. As per Mahua *et al*. (2021), the yellowness (b*) values exhibited a substantial decline with increasing concentrations of MOLE in the treated patties, in comparison to the control group. The reduction in the yellowness of patties is attributed to the presence of natural antioxidants found in MOLE.

The study shows that all concentrations of *Moringa oleifera* extract have noticeably higher L* values than the control after 1 hour, suggesting an increase in brightness. Nevertheless, the brightness of all samples gradually diminishes. The concentration of 2.0% consistently maintains the highest level of brightness (L*) when compared to other concentrations at later time intervals (24, 48, and 72 hours). The decrease in the L* values ($p < 0.05$) of the treated samples can be attributed to the reduced moisture content caused by the addition of Moringa oleifera leaf extract. This is due to the established correlation between moisture and lightness values, as documented by Pérez-Álvarez *et al*. (1999). Furthermore, the addition of *Moringa oleifera* leaf extract resulted in a reduction in the brightness of ground beef. This is due to the presence of chlorophyll, a green pigment found in *Moringa oleifera* leaf extract, which diluted the natural flesh pigment, hemoglobin, thereby affecting the color of the ground beef.

In the study conducted by Mashua *et al*. (2021), it was observed that the lightness (L*) values of the treated samples decreased significantly as the content of Moringa leaf extract increased, in comparison to the control group. Nyati (2017) found that the L*-values of ground beef samples treated with various quantities of *Moringa oleifera* leaf extract, broccoli extract, and sodium sulphite did not show any significant difference.

4.3 pH

The term "pH" refers to the measure of acidity or alkalinity of a solution. The pH level of ground beef is essential for determining its quality and ensuring its preservation. The inherent pH of fresh ground beef often lies within a somewhat acidic spectrum, approximately ranging from 5.5 to 6.0. At first, the inclusion of *Moringa* extract has no substantial impact on the pH level of the meat. Over time, the pH of all samples shows a small increase, suggesting a progressive transition towards alkalinity. Microbial and enzymatic activities commonly occur in beef preservation. The concentration of 2.0% exhibits the highest pH values at later time intervals, specifically at 48 and 72 hours. The application of *Moringa oleifera* leaf extract can aid in preserving optimal pH levels and prolonging the storage duration of ground beef. Madane *et al*.

(2019) also noted a rise in the pH of chicken nuggets that were supplemented with *Moringa oleifera* flower extract during the storage period.

4.4 Total Bacterial Count (TBC)

Total Bacterial Count (TBC) is a quantitative assessment of the whole microbial population present in ground beef. The control sample, which did not include a preservative, exhibited a microbial count above 105 colony-forming units per gram after being stored for 72 hours. This count serves as the arbitrary threshold for the shelf life "end point," at which indications indicative of deterioration become apparent (Steyn, 1989). The research conducted by Okorondu *et al.*, (2015) confirms that *Moringa oleifera* has strong antimicrobial properties. The study found that *Moringa oleifera* contains bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and cyanogenic glycosides, which, when used in appropriate amounts, can effectively inhibit and eliminate microorganisms. *Moringa oleifera* contains phenolic chemicals that can function as reducing agents and metal ion chelators when exposed to different hydroxyl radicals (Dorman and Deans, 2000).

During the first hour, the TBC values of all concentrations (0.5%, 1.0%, 1.5%, and 2.0%) of Moringa oleifera extract were lower than those of the control group. This suggests that the extract hinders the proliferation of all bacteria present in the meat. During the 72-hour storage period, the total bacterial count (TBC) steadily rises in all samples, as is anticipated due to the microbial degradation of meat. Significantly, the control group consistently demonstrates the highest Total Bacterial Count (TBC) values across all time intervals, suggesting that the Moringa extract possesses a preservation property that effectively inhibits bacterial proliferation. The 0.5% and 1.0% concentrations of Moringa extract exhibit superior efficacy in reducing bacterial growth when compared to higher concentrations and the control group.

The study conducted by Zhang *et al*. (2016) shown a significant reduction in the total viable count (TVC) of raw chicken flesh when spice extracts were added. In the study conducted by Jayawardana *et al*. (2015), chicken sausages that were treated with 0.5%, 0.75%, and 1% *Moringa oleifera* leaf extract showed significantly lower total phenolic content (TPC) values compared to chicken sausages treated with 0.25% MLE and the control sample. This difference was observed consistently over a storage duration of 5 weeks. The observed differences were statistically significant ($p < 0.05$). In a study conducted by Falowo *et al*. in 2016, it was shown that adding 1 g/kg of Moringa leaf extract (ethanolic-aqueous) to ground beef samples stored at 4 ◦C for 6 days resulted in a significant decrease in total viable counts compared to the control and samples treated with butylated hydroxytoluene (BHT). This decrease was observed as early as Day 3 of storage ($p < 0.05$). The results suggest that MLE has the potential to serve as a natural antibacterial agent in meat products. When the number of microorganisms in meat exceeds 7.00 log CFU/g of TVC, it indicates that the meat is spoiled and may pose health risks (ICMSF, 1986).

4.5 Total Coliform Count (TCC)

The Total Coliform Count (TCC) is a measure of the presence of coliform bacteria, which might indicate the presence of fecal pollution and inadequate hygiene (Martin *et al*., 2016). Like TBC, any concentration of *Moringa oleifera* extract leads to reduced TCC levels in comparison to the control during the initial stage (1 hour). Over the course of time, the total cell count (TCC) increases for all samples, however, the control group consistently demonstrates the highest TCC values. The *Moringa* extract with a concentration of 1.5% demonstrates the most notable decrease in TCC (total coliform count) when compared to other concentrations and the control group at all time intervals. These findings indicate that the *Moringa* extract effectively reduces the growth of coliform bacteria in ground beef by utilizing its antibacterial capabilities. The presence of polyphenolic components, including total phenolic content and total flavonoids content, in the extract is responsible for this impact (Mashua *et al*., 2021). The chemicals mentioned in the study by Jayawardana *et al*. (2015) help prevent the oxidation of lipids and the growth of microorganisms in beef.

In a study conducted by Rahman *et al*. (2020), it was found that the presence of total coliforms in goat meat nuggets decreased significantly ($p < 0.05$) when treated with 0.1%, 0.2%, and 0.3% MLE during frozen storage. This decrease was observed in comparison to both the control group and the goat meat nuggets treated with 0.1% butylated hydroxyanisole (BHA). In a study conducted by Mashau *et al*. (2021), it was observed that control mutton patties had a high coliform count of 6.20 log10 CFU/g at the end of the 15-day storage period. However, mutton patties treated with 1%, 2%, 3%, and 4% of MLE showed significantly lower coliform counts of 5.77, 4.88, 3.06, and 2.02 log10 CFU/g, respectively. The researchers also noted a significant increase in coliform counts in all treated samples throughout the 15-day storage period.

4.6 *Staphylococcus spp*

Staphylococcus spp refers to many species of bacteria belonging to the Staphylococcus genus.Staphylococcus is a bacterial genus comprising both pathogenic and non-pathogenic species. Certain Staphylococcus species have the potential to cause disease. At the initial stage (1 hour), the inclusion of Moringa extract led to reduced *Staphylococcus* counts in comparison to the control. Over a period of time, the number of *Staphylococcus* bacteria normally rose in all samples, but the control group typically exhibited greater numbers. The 0.5% concentration of *Moringa* extract exhibits the most efficacy in reducing *Staphylococcus* counts compared to other concentrations and the control group.These findings suggest that *Moringa* extract may have the ability to prevent the growth of *Staphylococcus* bacteria in ground beef. Moringa extract likely inhibits Staphylococcus growth through its rich content of bioactive compounds, such as phenolic compounds, flavonoids, and alkaloids. These compounds possess antimicrobial properties that interfere with the growth and reproduction of Staphylococcus bacteria by disrupting their cell membranes, inhibiting enzyme activity crucial for bacterial survival, and altering metabolic processes essential for growth.

As to Jayawardana *et al*. (2015), M. oleifera leaves contain pterygospermin, a chemical that readily breaks down into two molecules of benzyl isothiocyanate, a substance renowned for its antibacterial properties. According to the same authors, when chicken sausages were treated with *M. oleifera* leaves at concentrations of 0.25%, 0.5%, 0.75%, and 1%, the number of *S. aureus* bacteria per gram was less than 102 CFU. In addition, Elhadi *et al*. (2017) found that the levels of *S. aureus* were lower in refrigerated chicken patties treated with 100 g/kg MLP compared to chicken patties treated with 50 g/kg MLP and control patties (without treatment) over the 12-day storage period.

4.7 *Escherichia coli*

E. coli is a prevalent bacterium, and specific strains can be harmful, leading to foodborne diseases. At the initial stage (1 hour), the *Moringa* extract demonstrated a reduction in *E. coli* counts compared to the control. Over time, the number of *E. coli* bacteria varies, but both the control group and the samples treated with the extract show comparable patterns. The 0.5% concentration of *Moringa* extract exhibits the most notable decrease in E. coli counts when compared to other concentrations and the control group. This implies that although the extract may initially have inhibitory effects on *E. coli,* its influence may decrease over time.

The in vitro analysis of *M. oleifera* leaf extracts revealed that MLE had strong antibacterial properties against Gram-negative bacteria. Notably, the most significant inhibitory impact of the extracts was observed against *E. coli* (Falowo *et al*., 2015). The antibacterial action of *M. oleifera* leaves may be attributed to the low-weight proteins and peptides. In addition, the presence of E. coli was not detected in chicken sausages that were treated with *M. oleifera* leaves at concentrations of 0.25%, 0.5%, 0.75%, and 1% (Jayawardana *et al*., 2015). Furthermore, the concentration of *E.*

coli was found to be significantly higher in refrigerated chicken patties that did not contain *M. oleifera* leaf powder (MLP) compared to chicken patties that were treated with MLP, as observed in the study conducted by Elhadi *et al*. in 2017. Therefore, Maximum Likelihood Estimation (MLE) has the potential to be employed as a powerful antibacterial treatment for suppressing the growth of *E. coli* in meat products.

The current study demonstrates the vulnerability of *E. Coli* to the leaf extract of *M. oleifera*. This is consistent with the research conducted by Falowo *et al*. (2016), who observed that the extract from drumstick leaves had the most effective inhibitory impact on *E. coli*, resulting in a zone of inhibition with a diameter of 19 mm. In a similar vein, Adeyemi *et al*. (2013) documented the efficacy of *Moringa oleifera* extract in eradicating E. coli in fish. However, Rajendrhan *et al*. (1998) found that E. coli showed resistance to *Moringa oleifera* extracts, contradicting previous findings. Bhawasar *et al*. (1965) demonstrated that extracts of *Moringa oleifera* were not effective in combating E. coli. The disparity can be ascribed to discrepancies in the environmental conditions at the site of plant collection, the specific season, and the physiological phase of the plant during leaf harvesting (Taylor and Van Staden 2001), as these factors influence the chemical composition and quantity of chemicals present in the plant.

4.8 Sensory Evaluation

This study reveals a significant ($p<0.05$) improvement in ground beef color after *Moringa oleifera* extract treatment (2% control and 0.5%). Color scores were 5.3–7.3 on a 9-point scale. The control group was rated 7.30 for having the best color. As Moringa extract content grew, color evaluations decreased, suggesting that larger quantities may negatively affect meat color. Color preference increases significantly at 1.5% and 2% preservative concentrations compared to the control sample. At 0.5% and 1% concentrations, the change is negligible. Myoglobin affects meat color. Free radicals and aerobic bacteria cause methemoglobin to change color unfavorably (Renerre and Labas 1987). Drumstick leaf crude extracts can scavenge free radicals and preserve color (Sreelatha and Padma 2009). Hazra *et al*. (2012) found that ground buffalo meat treated with 1.5% extract had a substantial ($P < 0.05$) enhancement in color compared to other treatments.

According to this study ground beef texture improved significantly ($p<0.05$) by 2% compared to the control and 0.5%. Color scores were 4.7–7.0 on a 9-point scale. Preservatives improve texture preference at all concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control sample. As preservative concentration increases, texture preference improves. The control sample earned the best texture rating, like color. As *Moringa oleifera* extract concentration grew, texture scores fell. Hazra *et al.,* (2012) found that ground buffalo meat treated with 1.5% crude extract had a substantial ($p<0.05$) improvement in flavor score compared to other samples. This may be related to better lipid peroxidation inhibition.

Significant taste differences ($p<0.05$) were found in all research samples compared to the control sample. A considerable difference existed between 2%, 0.5%, and 1%. Color values varied from 3.0 to 7.9 on a 9-point scale. Taste preference increased significantly for all preservative concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control. Increased preservative content reduces taste. The control sample earned the best taste rating. Taste ratings dropped as *Moringa oleifera* extract content increased.

There is a significant difference ($p<0.05$) in juiciness between the control sample with 1%, 1.5%, and 2%. Color scores were 4.4–7.1 on a 9-point scale. Compared to the control sample, all preservative concentrations (0.5%, 1%, 1.5%, and 2%) showed a substantial increase in juiciness preference. Increased preservative concentration improves juiciness. Juiciness was highest for the control sample, suggesting it was the juiciest. The juiciness scores declined with higher *Moringa oleifera* extract concentrations. Hazra *et al.,* (2012) found substantial ($P<0.05$) differences between treated and control samples, but no significant difference ($P>0.05$). Rahman *et al.,* (2020) discovered that goat meat nuggets treated with 0.3% MLE had improved color, flavor, softness, juiciness, and overall acceptability during frozen storage ($p < 0.05$) compared to those treated with 0.1% BHA.

Significant flavor differences ($p<0.05$) were found in all research samples compared to the control sample. A considerable difference existed between 2%, 0.5%, and 1%. Color values varied from 3.1 to 7.7 on a 9-point scale. The preservatives increased flavor preference significantly at all concentrations (0.5%, 1%, 1.5%, and 2%) compared to the control. As preservative content rises, flavor preference decreases. Control sample earned the highest flavor rating, indicating it was the most preferred. The flavor ratings declined with higher concentrations of *Moringa oleifera* extract.

Hazra *et al.* (2012) found that ground buffalo meat treated with 1.5% crude extract had a substantial (P<0.05) improvement in flavor score compared to other samples. According to Abdallah *et al.,* 2023, beef meatballs treated with 0.5%, 1%, and 2% MLE showed considerable (p < 0.01) Moringa taste intensity during storage.

Ground beef quality and attractiveness depend on its overall acceptability. All study samples showed a significant difference (p<0.05) in taste compared to the control sample. A considerable difference existed between 2%, 0.5%, and 1%. Color scores varied from 3.7 to 7.7 on a 9-point scale. All concentrations (0.5%, 1%, 1.5%, and 2%) of preservatives showed a substantial increase in overall acceptability compared to the control. Increased preservative concentration decreases acceptability. High concentrations of Moringa extract (1.5% and 2.0%) impair sensory ratings across all criteria, showing that excessive amounts may lower meat sensory quality. Hazra *et al*. (2012) found a significant (P<0.05) improvement in overall acceptability scores, but no significant difference between treated samples. GBM treated with 1.5% scored higher than the other samples. Abdallah *et al.,* (2023) found no significant difference in beef meatball flavor, tenderness, juiciness, and acceptability between treated and control samples. However, treated meatball samples showed a slight improvement in tenderness and juiciness compared to the control.

4.9 Potential Cofounding Variables in the Study

Firstly, the variability in the microbial load of the initial ground beef samples could be a confounding factor. The presence of different levels of microbial contamination across samples could influence the observed antimicrobial efficacy of the *Moringa oleifera* leaf extract. To mitigate this, random sampling techniques and standardized microbial testing methods are to be employed to ensure consistency and reliability in the results. Secondly, the specific strains of microorganisms present in the ground beef samples may respond differently to the antimicrobial properties of the *Moringa oleifera* leaf extract. Without comprehensive identification and characterization of the microbial species involved, it may be challenging to ascertain the broad-spectrum effectiveness of the extract (Derbo & Debelew 2023). Including a diverse panel of bacterial strains representative of common foodborne pathogens would enhance the study's relevance and applicability to real-world scenarios.

Furthermore, the study may overlook potential interactions between *Moringa oleifera* leaf extract and other ingredients commonly used in ground beef formulations. Additives such as salt, spices, and preservatives could interact with the extract, altering its antimicrobial activity or overall efficacy in food preservation (Gandji *et al.,* 2020). Conducting additional experiments to assess the compatibility of the extract with typical food additives would provide valuable insights into its practical utility in food processing.

Moreover, the study's focus on ground beef alone may limit the generalizability of the findings to other food matrices. Different types of meat, poultry, or seafood products may exhibit varying levels of susceptibility to microbial contamination and respond differently to antimicrobial treatments. Consequently, extending the investigation to a broader range of food products would yield a more comprehensive understanding of the extract's applicability across diverse food systems.

Additionally, the study's reliance on laboratory-scale experiments may not fully capture the complexities of microbial growth and food spoilage dynamics under real-world storage conditions. Factors such as temperature fluctuations, oxygen exposure, and packaging materials could influence the efficacy of the *Moringa oleifera* leaf extract in preserving ground beef (Boopathi & Raveendran, 2021). . Therefore, conducting follow-up studies under simulated or actual storage conditions commonly encountered in the food industry would enhance the study's practical relevance and reliability.

4.10 Potential applications of the study’s findings in the food industry

The significant application of this study's findings is in the development of natural preservatives for ground beef products. Traditional preservatives often contain synthetic chemicals, raising concerns about their long-term health effects and environmental impact. The use of *Moringa oleifera* leaf extract offers a natural alternative, addressing consumer preferences for clean-label products while effectively inhibiting microbial growth. This aligns with the growing demand for natural and sustainable food preservation methods within the food industry (Grosshagauer *et al.,* 2021).

Moreover, the antimicrobial properties of *Moringa oleifera* leaf extract can help mitigate bacterial contamination during various stages of ground beef processing and distribution. From slaughterhouses to retail shelves, ground beef is vulnerable to pathogens such as Escherichia coli, Salmonella, and Listeria monocytogenes (Mehwish *et al.,* 2022) Incorporating *Moringa oleifera* leaf extract into processing facilities and packaging materials can serve as an additional barrier against microbial proliferation, reducing the risk of foodborne illnesses and product recalls.

The versatility of Moringa oleifera leaf extract also opens doors for innovative product development in the food industry. Beyond ground beef preservation, this natural antimicrobial agent could be explored in other meat products, dairy items, and even plant-based alternatives. Its broad spectrum of activity against various microorganisms provides manufacturers with a multifunctional ingredient for enhancing food safety and extending shelf life across a range of perishable goods (Hodas *et al.,* 2021).

Furthermore, the utilization of *Moringa oleifera* leaf extract supports sustainability initiatives within the food industry. Moringa trees are known for their rapid growth, resilience to adverse conditions, and minimal water requirements, making them an environmentally friendly crop. By harnessing the antimicrobial properties of *Moringa oleifera* leaf extract, food producers can reduce reliance on synthetic preservatives, minimize food waste, and contribute to more sustainable supply chains (Boopathi & Raveendran, 2021).

CHAPTER FIVE

5.0 GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 General conclusion

The study on the antimicrobial effect of *Moringa oleifera* leaf extract in ground beef preservation encountered various challenges, including assessing pH, total phenolic and flavonoid contents, foodborne pathogens, and color attributes. Variations in sample preparation and extraction techniques affected accuracy and reproducibility, necessitating standardization. Identification of pathogens faced challenges due to specialized techniques, while assessing color attributes had technical issues. Despite limitations, the study demonstrated that a 1.5% concentration of *Moringa oleifera* leaf extract improved taste, aroma, and overall acceptability of ground beef, with significant microbial reductions. While not affecting meat pH, the extract shows promise as an organic preservative, aligning with consumer demand for natural, health-conscious options and addressing food safety concerns. Further exploration of *Moringa oleifera's* potential could revolutionize meat preservation, enhancing sustainability and consumer well-being.

5.2 Recommendations

In view of the conclusions above, it is therefore recommended that:

- Investigate the possible synergistic outcomes of mixing *Moringa oleifera* leaf extract with additional natural preservatives or packaging technologies to improve the overall durability and quality of chilled ground beef products.

- Another study should be conducted to explore other extraction methods for *Moringa oleifera* and to enhance the concentration of *Moringa oleifera.*
- Collaboration with industry partners and regulatory agencies is necessary to develop guidelines and standards for including *Moringa oleifera* leaves extract in ground beef products. This collaboration will ensure that the products comply with food safety rules and labeling requirements.
- The antimicrobial properties of *Moringa oleifera* should be examined in various food items.
- A study on the antimicrobial mechanism of Moringa extract should be done.

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APPENDICES

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