

## Research Article

# Shelf-Life Feasibility Study of *Moringa oleifera* Seasoned Beef

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This study explored the stability of beef treated with *Moringa oleifera* ethanolic leaves extract (MELE) stored at 4°C for 10 days. Beef samples were treated with MELE concentrations of 8%, 4%, and 2%. A 3% NaCl-treated beef sample and control were included. Samples were taken every 48 hours for microbiological analysis. The Nordic Committee on Food Analysis methods were used to determine the mesophilic, coliform, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp. enumeration. The organoleptic properties were tested using the 5-point scale hedonism. There was a significant difference ( $p < 0.05$ ) in the mean mesophilic and coliform counts, which varied from  $2.0 \times 10^3 \pm 0.0$  to  $8.9 \times 10^7 \pm 0.4$  CFU/ml and  $1.0 \times 10^1 \pm 0.1$  to  $8.9 \times 10^4 \pm 0.3$  CFU/ml, respectively, from storage day two to ten when beef treated with MELE was compared to the 3% NaCl and the control. *Staphylococcus aureus* grew on all the beef samples across all the different incubation periods, but a dose-dependent antibacterial activity of the *M. oleifera*-treated beef was observed for *S. aureus* with the maximum inhibition recorded for 8% MELE. Beef seasoned with MELE was free of *E. coli* throughout storage. *Salmonella* sp. was not detected in all the beef samples. Microbial loads increased with decreasing concentrations of MELE. The overall acceptability of MELE-treated beef decreased with increasing concentration of MELE. MELE may find application in food preservation. The probability of synergistic activity of salt and *M. oleifera* leaves extract can be explored in further studies.

## 1. Introduction

Beef constitutes a vital component of food and dietary requirements in most developing countries [1]. Due to the increase in population, the demand for beef is on the rise [1]. Unfortunately, raw beef can easily perish if not handled well. For any food product, food safety is a top priority for the producers before the sensory quality. The capacity to keep food-borne microbes and pathogenic organisms below regulatory limits is crucial for extending the shelf life of beef because microorganisms are one of the significant variables that affect the stability of meat [2]. Studies have indicated that the bacterial load of raw meat in Ghana is very high due to the various contamination processes it undergoes from the slaughterhouse to the butcheries [3]. Contamination can lead to spoilage of beef and cause food-related illnesses.

The stability and shelf life of beef is ultimately influenced by the production process. For example, meat from the conventional market production system tends to have fewer food-borne pathogens than meat from the niche market production system [4]. Apart from preslaughter handling which may influence glycogen levels and hence pH of meat, spoilage of beef may occur as a result of postslaughter activities such as microbial contamination, lipid oxidation, and autolysis [5]. Factors, such as the physiological state of the animal at the time of slaughtering, the microbial contamination in the areas of handling, the hygiene practices of the handlers, and the level of contamination of the tools used, may all contribute to the initial microbial load on meat [6] and hence its final stability.

Although raw beef is readily available to the wealthy and middle-class consumers in major cities, the majority of impoverished people may not have the means of keeping it

fresh. Therefore, many cannot easily access beef due to stability challenges. To keep raw beef fresh, it needs to be frozen. However, due to power instability in most developing countries [7], there is a need to find alternative ways of keeping beef fresh. Artificial preservatives have been used over decades to keep beef stable for a long time. However, these have been associated with health problems, such as cancer, mental retardation, nausea, weakness, and headaches [8]. Also, synthetic preservatives tend to reduce the nutritional quality of meat [9]. For these reasons, consumers prefer naturally preserved meat products with prolonged shelf life. The alternatives to increasing the nutritional value and stability of foods against the use of artificial preservatives are plant-based extracts. There have been studies using plant-based extracts to increase the shelf life of meat products [10–14]. One such plant extract which has no known negative side effects is *Moringa oleifera* [15–18].

*M. oleifera* is effective against a wide range of food-spoilage bacteria and fungi. It has been reported that *Moringa* leaves extracts with its reasonable safety margins may be used as an antimicrobial agent to inhibit bacterial growth in food [19]. This includes those that cause gastric ulcers and gastric cancer. *M. oleifera* leaf extracts can reduce the microbial load of treated meat under cold conditions [20] and may also act as a natural antioxidant to inhibit lipid oxidation in some meat products [21]. Apart from this, it also contains phenolic compounds which possess anti-inflammatory, antioxidant, neuroprotective, and hepatoprotective activities [22] which can reduce the risk of cardiovascular diseases and diabetes [23].

Although there are a lot of factors that have an impact on the stability and shelf life of beef, preservation methods that can ensure the quality and stability of meat may contribute to the reduction of food insecurity. Therefore, the objective of this study was to determine the stability of beef treated with *M. oleifera* leaf extract as an alternative preservation method.

## 2. Materials and Methods

**2.1. Sample Collection.** Fresh beef of 2 kg was acquired from the Makola market in the Greater Accra region in duplicate. The meat was aseptically transported to the Microbiology Laboratory at a cold condition of 4°C. This was washed twice under running water and deboned. All connective tissues and fats were detached and the beef was frozen at –18°C until usage. Certified leaves of *M. oleifera* were obtained from an indigenous farmstead in Kasoa in the Central Region of Ghana. These were aseptically washed and dried in a Genius food dehydrator Ksh 6500 at 30°C.

**2.2. *Moringa oleifera* Extraction with Ethanol.** The dried *M. oleifera* leaves were blended using an electric grinder (Binatone BLG-450 MKS) into a fine powder (100 g), dissolved in 500 ml of ethanol, and incubated at room temperature for 72 hours with stirring at regular intervals. The extracts were filtered through Whatman No. 41 filter paper and the filtrates were vacuum-dried with the rotary evaporator to achieve a concentrated mixture.

**2.3. Preparation of Samples.** The beef was thawed and cut into 50 g pieces under refrigeration conditions (3 to 4°C). These were subjected to 5 different treatments: 3% NaCl, 2% *M. oleifera*, 4% *M. oleifera*, 8% *M. oleifera*, and control. These were sealed in airtight Ziploc bags and stored at a temperature of 4°C for 10 days. Microbiological analysis was performed on the samples in duplicates every 48 hours.

**2.4. Microbiological Analysis.** The analyses of mesophilic and coliform counts, as well as the identification and enumeration of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp., were conducted using the procedures outlined by the Nordic Committee on Food Analysis (NMKL). Ten (10) grams of each beef sample were serially diluted ( $10^{-1}$  to  $10^{-6}$ ) in tenfolds using sterile saline water. A homogenized suspension of each sample was prepared in a stomacher bag by mixing for two minutes and an aliquot (1 ml) of each suspension was plated. Further analyses were performed as follows.

**2.5. Mesophilic Count Determination.** The NMKL No 86 protocol was employed in the determination of the mesophilic count. Sterile Petri dishes were filled with 1 ml aliquots of each series of dilutions and molten Plate Count Agar (PCA). After gently swirling to form even mixtures, each was allowed to solidify. These were incubated at 30°C for 48 hours and the formed colonies were counted [24].

**2.6. Coliform Determination.** The NMKL No 44 protocol was employed in the determination of the coliform count. A 1 ml aliquot of each dilution was transferred into a sterile Petri dish and molten Violet Red Bile Agar was added. Each mixture was swirled to form a homogeneous suspension and allowed to solidify. These were incubated for 24 hours at 37°C and 44.5°C. Enumeration was performed for the red colonies that were formed. In Brilliant Green Bile Broth and *Escherichia coli* Broth, the presence of the colonies was confirmed by gas production [24, 25].

**2.7. *Staphylococcus aureus* Determination.** The NMKL No 66 protocol was employed in the enumeration of *Staphylococcus aureus*. Petri dishes containing molten Baird-Parker Agar were filled with aliquots of each dilution and swirled to obtain even mixtures. These were incubated at a temperature of 37°C for 24 hours. *Staphylococcus aureus* were counted and confirmed by coagulase-positive test [26].

**2.8. Determination of *Escherichia coli*.** The NML No 125 protocol was employed in the enumeration of *Escherichia coli*. Samples from the positive *Escherichia coli* Broth tubes were transferred into tryptone water and incubated at 44°C for 48 hours. Indole tests were performed by adding 3 drops of Kovac's reagent to each test culture. The formation of a red color indicated the presence of *Escherichia coli* [27] and

the enumeration was carried out by using the most probable number (MPN).

**2.9. *Salmonella* spp. Detection.** The NMKL No 71 protocol was employed in the identification and enumeration of *Salmonella* spp. by homogenizing 25 g of each beef sample in a stomacher bag with 225 ml of saline water and incubating each for 24 hours at a temperature of 37°C. Aliquots of 1 ml were transferred into 10 ml of tetrathionate broth base and incubated at 37°C for 24 hours. Also, 0.1 ml of each inoculum was transferred into Rappaport-Vassiliadis Soya Peptone Broth (10 ml) and incubated for 24 hours at 41.5°C. Colonies from both media were plated on Bismuth Sulfite Agar and incubated at 37°C. These were examined after 24 hours [28].

**2.10. Sensory Analysis.** The treated beef samples were boiled for 25 minutes and allowed to cool at room temperature during each storage day (2, 4, 6, 8, and 10 days). The 5-point hedonic scale (1 = dislike very much, 2 = dislike slightly, 3 = neither like nor dislike, 4 = like slightly, and 5 = like very much) was used to determine the organoleptic properties of the samples: thirty (30) untrained panel were used for the evaluation of the meat samples in terms of color, aroma, texture, taste, and overall acceptability. After tasting each meat sample, the panelists were given portable drinking water for mouth rinsing.

**2.11. Statistical Analysis.** The Minitab statistical software version 21 was used for the two-way ANOVA and comparisons among treatments and interactions with the storage. The mean data  $\pm$  SD (standard deviation) was computed. At  $p < 0.05$ , differences between means were statistically significant.

### 3. Results

All the beef samples had mesophilic counts ranging from  $2.0 \times 10^3$  CFU/ml to  $8.9 \times 10^7$  CFU/ml from 2 to 10 days of storage (Table 1). Analysis of variance showed a significant difference within the beef treated with 3% NaCl. Multiple comparison tests with beef seasoned with MELE and 3% NaCl showed no significant difference ( $p > 0.05$ ) from the 2<sup>nd</sup> to 8<sup>th</sup> day of storage. However, when compared to the control, there was a significant difference ( $p \leq 0.05$ ) from the 2<sup>nd</sup> to the 10<sup>th</sup> day of storage. The mesophilic counts increased with decreased concentration of *Moringa* leaf extract, while it increased during storage from day 2. Also, the mesophilic count was decreased in the MELE-seasoned beef when compared to the 3% NaCl-cured beef. The control recorded the highest microbial load during storage from day 2.

The total coliform growth (Table 2) was significantly higher ( $p < 0.05$ ) in the control than in the treated samples. Multiple comparison tests showed that the total plate counts for beef seasoned with MELE, and 3% NaCl were not significantly different ( $p > 0.05$ ) during storage from the second to the fourth day. For all treatments, the coliform counts

decreased from day 0 to day 2. However, for all the treatments there was an increase in coliforms from day 2 to day 10. Also there was a decrease in coliform on days 8 and 10 for 3% NaCl and 2% MELE. The coliform count was lower in the MELE beef treatments than in the beef cured with 3% NaCl from day 2 to 10.

*Staphylococcus aureus* grew on all the beef samples (Table 3). The control recorded higher levels ranging from  $2.0 \times 10^4 \pm 0.2$  to  $7.0 \times 10^5 \pm 0.3$  from day 2 to 10. Growth was observed across all the different incubation periods, but a dose-dependent antibacterial activity of the *M. oleifera*-treated beef was observed for *S. aureus* with the maximum inhibition recorded for 8% MELE-beef treated samples.

There was no growth of *Escherichia coli* in all the MELE-treated beef samples throughout the storage period (Table 4).

None of the samples contained *Salmonella* spp. (Table 5).

Information on the products' taste, aroma, appearance, texture, and overall acceptability is exhibited in Table 6. The sensory parameters were graded on a scale of 1 to 5.

**3.1. Taste.** The investigation on taste revealed a clear distinction between the various beef treatments. There were significant differences ( $p > 0.05$ ) for samples treated with 8% MELE extract. However, there was no discernible variation in taste between the samples on day 0 and day 2 ( $p > 0.05$ ). Similarly, there were no significant changes for samples on days 4, 6, 8, and 10. At 4% treatment levels, there were significant variations in the storage days ( $p > 0.05$ ). However, there were no variations between the samples collected on days 0 and 2. While samples on days 0, 2, 4, and 6 did not reveal any significant differences ( $p > 0.05$ ), treatments at 2% did indicate significant differences ( $p > 0.05$ ). There were no substantial alterations for samples held for 0, 4, 6, or 10 days and for samples treated with 3% NaCl.

The study, however, indicated that there were no differences between control samples and samples treated with 8% MELE and stored for 6 days, 8 days, and 10 days, respectively. Results also showed that the taste of the control samples at days 0, 2, and 4 was liked by the sensory panel. However, the taste of the samples stored for 4, 6, 8, and 10 days as well as control samples for 6, 8, and 10 days were not liked by the panel.

**3.2. Aroma.** There were no differences between the days of storage and samples treated with 8% MELE, 4% MELE, and 3% NaCl ( $p > 0.05$ ). However, the samples treated at 2% MELE and the storage days showed significant variations ( $p > 0.05$ ). Moreover, storage days and the control samples showed statistically significant differences ( $p > 0.05$ ). The study's findings revealed that samples treated at 2% MELE had the highest ratings for aroma. The panel did not like the samples' aroma after the 8% MELE treatment. The control samples at days 0 and 2 were, however, liked by the panel.

**3.3. Appearance.** The analysis of variance in appearance revealed that the sample treatment and storage days had a significant impact ( $p > 0.05$ ). The study's findings also

TABLE 1: Mean CFU/ml of total plate counts of raw beef seasoned with *Moringa oleifera* leaves extract (MELE) during storage.

Storage (days)	Control	3% NaCl	2% MELE	4% MELE	8% MELE
0	$3.0 \times 10^6 \pm 0.0^B$	$3.0 \times 10^6 \pm 0.0^B$	$3.0 \times 10^6 \pm 0.0^B$	$3.0 \times 10^6 \pm 0.0^B$	$3.0 \times 10^6 \pm 0.0^B$
2	$2.0 \times 10^6 \pm 0.0^B$	$6.5 \times 10^3 \pm 0.0^C$	$4.8 \times 10^3 \pm 0.3^C$	$3.8 \times 10^3 \pm 0.0^C$	$2.0 \times 10^3 \pm 0.0^C$
4	$4.1 \times 10^7 \pm 0.1^A$	$7.0 \times 10^3 \pm 0.1^C$	$5.7 \times 10^3 \pm 0.0^C$	$4.5 \times 10^3 \pm 0.0^C$	$3.1 \times 10^3 \pm 0.0^C$
6	$5.1 \times 10^7 \pm 0.3^A$	$5.0 \times 10^4 \pm 0.0^C$	$3.3 \times 10^4 \pm 0.0^C$	$2.6 \times 10^4 \pm 0.1^C$	$1.1 \times 10^4 \pm 0.0^C$
8	$7.7 \times 10^7 \pm 0.3^A$	$7.0 \times 10^4 \pm 0.0^C$	$5.4 \times 10^4 \pm 0.0^C$	$4.4 \times 10^4 \pm 0.0^C$	$2.4 \times 10^4 \pm 0.0^C$
10	$8.9 \times 10^7 \pm 0.4^A$	$6.1 \times 10^6 \pm 0.0^B$	$8.5 \times 10^5 \pm 0.1^C$	$5.5 \times 10^4 \pm 0.0^C$	$4.0 \times 10^4 \pm 0.1^C$

Values are means  $\pm$  standard deviation of duplicate determinations. Means that do not share the same letter are significantly different ( $p \leq 0.05$ ).

TABLE 2: Mean CFU/ml of the total coliform count of raw beef seasoned with *Moringa oleifera* leaves extract (MELE) during storage.

Storage (days)	Control	3% NaCl	2% MELE	4% MELE	8% MELE
0	$5.0 \times 10^3 \pm 0.0^B$	$5.0 \times 10^3 \pm 0.0^B$	$5.0 \times 10^3 \pm 0.0^B$	$5.0 \times 10^3 \pm 0.0^B$	$5.0 \times 10^3 \pm 0.0^B$
2	$2.2 \times 10^3 \pm 0.0^B$	$4.2 \times 10^1 \pm 0.0^C$	$3.5 \times 10^1 \pm 0.1^C$	$2.0 \times 10^1 \pm 0.0^C$	$1.0 \times 10^1 \pm 0.1^C$
4	$4.1 \times 10^4 \pm 0.0^A$	$7.5 \times 10^1 \pm 0.1^C$	$5.0 \times 10^1 \pm 0.0^C$	$3.2 \times 10^1 \pm 0.0^C$	$1.8 \times 10^1 \pm 0.1^C$
6	$5.6 \times 10^4 \pm 0.3^A$	$5.6 \times 10^2 \pm 0.0^B$	$8.6 \times 10^1 \pm 0.0^C$	$4.6 \times 10^1 \pm 0.0^C$	$2.2 \times 10^1 \pm 0.0^C$
8	$6.8 \times 10^4 \pm 0.2^A$	$8.0 \times 10^2 \pm 0.0^B$	$9.5 \times 10^1 \pm 0.0^C$	$6.1 \times 10^1 \pm 0.1^C$	$3.0 \times 10^1 \pm 0.0^C$
10	$8.9 \times 10^4 \pm 0.3^A$	$6.6 \times 10^2 \pm 0.0^B$	$7.0 \times 10^1 \pm 0.0^C$	$6.1 \times 10^1 \pm 0.0^C$	$4.5 \times 10^1 \pm 0.1^C$

Values are means  $\pm$  standard deviation of duplicate determinations. Means that do not share the same letter are significantly different ( $p \leq 0.05$ ).

TABLE 3: Mean (CFU/ml) of *Staphylococcus aureus* on raw beef seasoned with MELE.

Storage (days)	Control	3% NaCl	2% MELE	4% MELE	8% MELE
0	$1.5 \times 10^3 \pm 0.0$	$1.5 \times 10^3 \pm 0.0$	$1.5 \times 10^3 \pm 0.1$	$1.5 \times 10^3 \pm 0.0$	$1.5 \times 10^3 \pm 0.1$
2	$2.0 \times 10^4 \pm 0.2$	$1.0 \times 10^2 \pm 0.0$	$6.1 \times 10^1 \pm 0.0$	$3 \times 10^1 \pm 0.0$	$1 \times 10^1 \pm 0.0$
4	$3.0 \times 10^4 \pm 0.0$	$2.0 \times 10^2 \pm 0.0$	$1.6 \times 10^2 \pm 0.1$	$7.0 \times 10^1 \pm 0.0$	$3.1 \times 10^1 \pm 0.0$
6	$4.5 \times 10^4 \pm 0.6$	$4.6 \times 10^2 \pm 0.0$	$2.8 \times 10^2 \pm 0.0$	$1.3 \times 10^2 \pm 0.0$	$7.5 \times 10^1 \pm 0.0$
8	$3.9 \times 10^4 \pm 0.01$	$7.4 \times 10^2 \pm 0.0$	$5.0 \times 10^2 \pm 0.0$	$2.4 \times 10^2 \pm 0.0$	$1.1 \times 10^2 \pm 0.0$
10	$7.0 \times 10^5 \pm 0.3$	$9.9 \times 10^2 \pm 0.0$	$8.0 \times 10^2 \pm 0.0$	$4.3 \times 10^2 \pm 0.0$	$2.0 \times 10^2 \pm 0.0$

Values are means  $\pm$  standard deviation of duplicate determinations.

TABLE 4: Mean (CFU/ml) of *Escherichia coli* on raw beef seasoned with MELE.

Storage (days)	Control	3% NaCl	2% MELE	4% MELE	8% MELE
0	$1.0 \times 10^3 \pm 0.0$	$1.0 \times 10^3 \pm 0.0$	$1.0 \times 10^3 \pm 0.0$	$1.0 \times 10^3 \pm 0.0$	$1.0 \times 10^3 \pm 0.0$
2	$3.0 \times 10^3 \pm 0.0$	$8 \times 10^1 \pm 0.1$	ND	ND	ND
4	$4.7 \times 10^4 \pm 0.0$	$1.6 \times 10^2 \pm 0.0$	ND	ND	ND
6	$5.5 \times 10^4 \pm 0.4$	$2.5 \times 10^2 \pm 0.0$	ND	ND	ND
8	$6.6 \times 10^4 \pm 0.1$	$3.6 \times 10^3 \pm 0.2$	ND	ND	ND
10	$8.3 \times 10^4 \pm 0.0$	$4.5 \times 10^3 \pm 0.0$	ND	ND	ND

Values are means  $\pm$  standard deviation of duplicate determinations. ND, not detected.

TABLE 5: Mean (CFU/ml) of *Salmonella* sp. on raw beef seasoned with MELE.

Storage (days)	Control	3% NaCl	2% MELE	4% MELE	8% MELE
0	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND

ND: not detected.

TABLE 6: Organoleptic properties of cooked beef seasoned with MELE.

Sensory parameter	Samples	0 day	2 days	4 days	6 days	8 days	10 days
Taste	Control	5.0 ± 0.1 <sup>A</sup>	5.0 ± 0.5 <sup>A</sup>	4.5 ± 0.1 <sup>AB</sup>	1.0 ± 0.0 <sup>I</sup>	1.0 ± 0.1 <sup>I</sup>	1.0 ± 0.4 <sup>I</sup>
	3% NaCl	3.0 ± 0.5 <sup>C</sup>	2.9 ± 0.0 <sup>CD</sup>	3.05 ± 0.2 <sup>C</sup>	3.1 ± 0.1 <sup>C</sup>	2.6 ± 0.2 <sup>D</sup>	3.0 ± 0.1 <sup>C</sup>
	2% MELE	3.6 ± 0.1 <sup>B</sup>	3.6 ± 0.0 <sup>B</sup>	3.6 ± 0.3 <sup>B</sup>	3.6 ± 0.1 <sup>B</sup>	3.1 ± 0.3 <sup>C</sup>	2.5 ± 0.6 <sup>D</sup>
	4% MELE	1.9 ± 0.1 <sup>EF</sup>	1.8 ± 0.2 <sup>EF</sup>	1.0 ± 0.1 <sup>I</sup>	2.0 ± 0.4 <sup>E</sup>	1.6 ± 0.5 <sup>EF</sup>	1.3 ± 0.0 <sup>GHI</sup>
	8% MELE	1.2 ± 0.5 <sup>HI</sup>	1.2 ± 0.0 <sup>HI</sup>	1.0 ± 0.2 <sup>I</sup>	1.0 ± 0.2 <sup>I</sup>	1.0 ± 0.0 <sup>I</sup>	1.0 ± 0.0 <sup>I</sup>
Aroma	Control	5.0 ± 0.6 <sup>A</sup>	5.0 ± 0.5 <sup>A</sup>	3.2 ± 0.1 <sup>C</sup>	1.1 ± 0.4 <sup>G</sup>	1.0 ± 0.2 <sup>G</sup>	1.0 ± 0.1 <sup>G</sup>
	3% NaCl	3.5 ± 0.0 <sup>B</sup>	3.5 ± 0.3 <sup>B</sup>	3.8 ± 0.6 <sup>B</sup>	3.8 ± 0.1 <sup>B</sup>	3.4 ± 0.4 <sup>BCD</sup>	3.8 ± 0.1 <sup>B</sup>
	2% MELE	3.5 ± 0.0 <sup>B</sup>	3.4 ± 0.1 <sup>BC</sup>	3.0 ± 0.0 <sup>D</sup>	3.0 ± 0.2 <sup>CD</sup>	3.0 ± 0.0 <sup>D</sup>	2.5 ± 0.2 <sup>E</sup>
	4% MELE	1.9 ± 0.2 <sup>F</sup>	1.9 ± 0.1 <sup>F</sup>	1.8 ± 0.2 <sup>F</sup>	1.7 ± 0.4 <sup>F</sup>	1.8 ± 0.0 <sup>F</sup>	1.8 ± 0.2 <sup>F</sup>
	8% MELE	1.0 ± 0.1 <sup>G</sup>	1.0 ± 0.1 <sup>G</sup>	1.0 ± 0.1 <sup>G</sup>	1.0 ± 0.0 <sup>G</sup>	1.0 ± 0.0 <sup>G</sup>	1.0 ± 0.2 <sup>G</sup>
Appearance	Control	5.0 ± 0.5 <sup>A</sup>	4.9 ± 0.1 <sup>A</sup>	3.5 ± 0.1 <sup>C</sup>	1.0 ± 0.6 <sup>I</sup>	1.0 ± 0.2 <sup>I</sup>	1.0 ± 0.5 <sup>I</sup>
	3% NaCl	4.0 ± 0.3 <sup>B</sup>	4.0 ± 0.1 <sup>B</sup>	4.0 ± 0.0 <sup>B</sup>	2.6 ± 0.1 <sup>E</sup>	2.3 ± 0.2 <sup>F</sup>	3.0 ± 0.5 <sup>D</sup>
	2% MELE	4.2 ± 0.2 <sup>B</sup>	4.0 ± 0.1 <sup>B</sup>	4.0 ± 0.4 <sup>B</sup>	3.4 ± 0.5 <sup>C</sup>	2.1 ± 0.0 <sup>FG</sup>	4.0 ± 0.0 <sup>B</sup>
	4% MELE	2.3 ± 0.3 <sup>F</sup>	2.0 ± 0.1 <sup>G</sup>	1.9 ± 0.4 <sup>G</sup>	2.0 ± 0.2 <sup>G</sup>	2.0 ± 0.4 <sup>G</sup>	2.0 ± 0.4 <sup>G</sup>
	8% MELE	1.4 ± 0.5 <sup>H</sup>	1.4 ± 0.1 <sup>H</sup>	1.0 ± 0.3 <sup>I</sup>	1.0 ± 0.2 <sup>I</sup>	1.0 ± 0.4 <sup>I</sup>	1.0 ± 0.3 <sup>I</sup>
Texture	Control	5.0 ± 0.3 <sup>A</sup>	4.5 ± 0.1 <sup>BC</sup>	3.3 ± 0.1 <sup>FGH</sup>	2.1 ± 0.1 <sup>J</sup>	1.0 ± 0.1 <sup>K</sup>	1.0 ± 0.3 <sup>K</sup>
	3% NaCl	4.9 ± 0.4 <sup>A</sup>	4.9 ± 0.6 <sup>AB</sup>	5.0 ± 0.1 <sup>A</sup>	5.0 ± 0.1 <sup>A</sup>	5.0 ± 0.1 <sup>A</sup>	3.4 ± 0.1 <sup>FGH</sup>
	2% MELE	4.3 ± 0.5 <sup>CD</sup>	4.2 ± 0.6 <sup>CD</sup>	4.0 ± 0.1 <sup>DE</sup>	3.3 ± 0.1 <sup>FGH</sup>	2.8 ± 0.2 <sup>I</sup>	2.2 ± 0.3 <sup>J</sup>
	4% MELE	3.6 ± 0.2 <sup>EF</sup>	3.5 ± 0.5 <sup>FG</sup>	3.6 ± 0.1 <sup>EF</sup>	3.6 ± 0.1 <sup>EF</sup>	3.6 ± 0.1 <sup>EF</sup>	2.2 ± 0.3 <sup>J</sup>
	8% MELE	3.5 ± 0.2 <sup>FG</sup>	3.6 ± 0.5 <sup>EF</sup>	3.4 ± 0.6 <sup>FGH</sup>	3.1 ± 0.1 <sup>HI</sup>	3.3 ± 0.2 <sup>FGH</sup>	2.2 ± 0.0 <sup>J</sup>
Overall acceptability	Control	5.0 ± 0.3 <sup>A</sup>	5.0 ± 0.2 <sup>A</sup>	4.1 ± 0.6 <sup>B</sup>	1.0 ± 0.5 <sup>I</sup>	1.0 ± 0.4 <sup>I</sup>	1.0 ± 0.3 <sup>I</sup>
	3% NaCl	4.5 ± 0.1 <sup>B</sup>	4.1 ± 0.2 <sup>B</sup>	3.8 ± 0.5 <sup>B</sup>	3.0 ± 0.4 <sup>C</sup>	2.2 ± 0.5 <sup>DE</sup>	2.3 ± 1.0 <sup>DE</sup>
	2% MELE	4.0 ± 0.6 <sup>B</sup>	3.9 ± 0.1 <sup>B</sup>	4.0 ± 0.2 <sup>B</sup>	3.1 ± 0.9 <sup>C</sup>	2.4 ± 0.6 <sup>DE</sup>	2.5 ± 0.7 <sup>D</sup>
	4% MELE	2.4 ± 0.6 <sup>D</sup>	2.0 ± 0.3 <sup>EF</sup>	2.5 ± 1.0 <sup>D</sup>	2.2 ± 0.9 <sup>DE</sup>	2.5 ± 0.3 <sup>D</sup>	1.7 ± 0.2 <sup>FGH</sup>
	8% MELE	1.7 ± 0.0 <sup>FG</sup>	1.7 ± 0.5 <sup>FG</sup>	1.4 ± 0.3 <sup>GHI</sup>	1.2 ± 0.4 <sup>I</sup>	1.3 ± 0.2 <sup>HI</sup>	1.1 ± 0.5 <sup>I</sup>

Values are expressed as means. Values within interactions of individual sensory attributes that do not share a letter are significantly different ( $p < 0.05$ ).

demonstrated that the panel did not like the way the samples treated at 8% MELE looked. On the other hand, the panel preferred the control samples on days 0 and 2. The results demonstrated that the panel appreciated samples that were given 2% MELE treatment on days 0, 2, 4, and 10. The sensory panel also preferred samples that had received 3% NaCl treatments on days 2 and 4.

**3.4. Texture.** The texture of the samples varied significantly ( $p > 0.05$ ) depending on the different treatments and storage days. The research revealed that samples treated with 8% and 4% MELE were preferred by the panel. Nonetheless, the panel preferred the samples that had been exposed to 2% MELE on days 0, 2, and 4. Except for samples on day 10, all samples that were treated with 3% NaCl were well-liked by the panel. The panel favored the texture of the control sample on day 0 but disliked the control samples' texture on days 8 and 10.

**3.5. Overall Acceptability.** Significant variations ( $p > 0.05$ ) between the sample treatments and the storage days were present. The panel did not like any of the products treated with 8% and 4% *Moringa*. The panel preferred samples that received 2% MELE treatment on days 0 and 4. The panel also preferred the control sample on days 0, 2, and 4. On days 6, 8, and 10, the panel did not like the control samples.

## 4. Discussion

This work focused on the microbiological and organoleptic quality of beef seasoned with *Moringa oleifera* leaves extract during storage and has shown that MELE exhibits anti-bacterial activity which can be exploited in food systems without adversely affecting the sensory attributes if used in relatively lower concentrations. Higher concentrations of MELE can be used to achieve a greater activity against food-spoilage bacteria without compromising safety since *M. oleifera* has been proven to have low toxicity. Intake has been proven to be safe at supra-supplementation levels  $\leq 1,000$  mg/kg, which is far below the quantity tested in this study, when used as a nutraceutical [29].

Generally, although the microbial load increased over the storage period from the 2<sup>nd</sup> day of storage, treated beef samples had relatively lower microbial loads as well as lower coliform counts than the control samples. Also, MELE-treated samples had relatively lower microbial loads than samples treated with 3% NaCl, especially at higher concentrations. The higher the concentration of the MELE, the slower the multiplication of the microorganisms in the samples during the storage and analysis period. The effect of MELE on the microbial load can be attributed to the fact that *Moringa oleifera* leaves are rich in various phytochemical compounds including glucosinolates [30] and polyphenols such as flavonoids, tannins, and phenolic acids [31]. Polyphenols are known to demonstrate broad-spectrum

antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungi [32]. Although the mechanism of action of polyphenols against bacteria has not been fully explained, the antibacterial activity has been attributed to a disruption of the bacterial cell membrane [33]. Similar, results have been reported by Mhalla et al. [11] which showed that the addition of extract from *Rumex tinctorius* to raw bovine minced meat retarded the growth of mesophilic and psychrophilic bacteria. In addition, the same study showed that the shelf life of pork sausage was enhanced when treated with the extract obtained from *Citrus* sp. [34].

Higher concentrations of MELE kept the growth of *Staphylococcus aureus* in beef at relatively lower microbial loads over the storage and analysis period as was observed in the total plate counts. The reduced *Staphylococcus aureus* load after treatment of the meat with MELE suggested that the MELE can be used as a potent organic plant extract for the preservation of meat and meat products against this bacteria. However, MELE appeared to be less effective in reducing the growth of *Staphylococcus aureus* when compared to its efficacy against *Escherichia coli*. Similarly, Bouarab-Chibane et al. [33] have shown that polyphenols exhibit a species-dependent antibacterial activity on Gram-negative and Gram-positive bacteria. The differences in susceptibility of *Staphylococcus aureus* and *Escherichia coli* to the MELE may be attributed to the differences in the hydrophobicity of their cell wall [27]. The surface charge of Gram-negative bacteria has been reported to be less negative in the presence of the two phenolic acids, while that of Gram-positive bacteria remains unchanged [28]. Although this may be the case, studies have shown that plant extracts can effectively abrogate the growth of not only food-borne microorganisms such as *Listeria monocytogenes*, *Pseudomonas* sp., and fungi but also *Staphylococcus aureus* [21–23]. This implies that MELE concentration above 8% may be required to significantly reduce the growth of some food-borne pathogens such as *Staphylococcus aureus* on beef during storage.

MELE effectively eliminated the growth of *Escherichia coli* in the beef samples during the storage and analysis period. The current observation is in concordance with the study by Zhao et al. [35] in which the addition of *Perilla frutescens* leaf extract significantly reduced the growth of *Escherichia coli* in surimi fish balls during storage at 4°C. Roila et al. [26] also reported a similar finding in which the incorporation of olive mill wastewater into cheese retarded the growth of Enterobacteriaceae and resulted in a significant extension of the shelf life. The observed progressive increase in the population of *Escherichia coli* over the 10-day incubation period for both the control and the NaCl-treated meat samples suggests that MELE may improve the shelf life of meat samples more than NaCl, especially in situations where *Escherichia coli* has been identified as the major contaminant during production. Food-derived polyphenols are natural preservatives and are less likely to cause the side effects associated with synthetic preservatives [20]. It may also serve as a replacement for preserving meat for those on salt-free diets. Apart from this, MELE may also

have a possible application in the treatment of meat before cold storage as it may potentially contribute to a reduction in the activity of psychrophilic and psychrotrophic bacteria during preservation as well as in the process of thawing before use.

The significant impact of beef seasoned with MELE on the flavor, aroma, appearance, texture, and general acceptability when compared to the control and 3% NaCl confirms findings by Rahman et al. [20] which indicated that goat meat nuggets treated with 0.3% MELE during frozen storage had significantly ( $p > 0.05$ ) improved color, flavor, softness, juiciness, and overall acceptability compared to the control and other goat meat nuggets treated with 0.1% butylated hydroxyanisole (BHA). The 8% MELE-seasoned beef was the least well-liked beef product while the 2% MELE was highly favored. This corresponds to investigations carried out by Evivie et al. [17] which revealed that soy meatballs treated with higher concentrations of *M. oleifera* leaves powder were not accepted by the panelist. However, the meatballs with lower concentrations of *M. oleifera* powder were accepted. Additionally, the addition of *M. oleifera* leaf powder to chicken patties up to concentrations of 50 g/kg did not affect the overall acceptability and other sensory parameters of chicken patties. However, concentrations above the 50 g/kg had the opposite effect [18].

The findings of this study concur with those of Abdallah et al. [16]. In their study, samples of beef that had been treated with 2% *Moringa* sp. received top marks for scent and flavor. *M. oleifera* leaves are abundant in polyphenols, carotenoids, flavonoids, and other bioactive compounds that enhance food flavor and aroma [36]. Nevertheless, large amounts of these components are unpleasant, as found in pito samples by Ayirezang et al. [15]. Although increased concentrations of MELE will have a favorable effect on reducing and eliminating food-borne microorganisms, sensory-wise lower concentrations are preferred.

## 5. Conclusion

MELE exhibits antibacterial activity which can be exploited in food preservation without adversely affecting the sensory attributes if used in relatively lower concentrations. Also, it has the potential to be used as a food preservative against meat spoilage associated with *Escherichia coli* but may be less effective against *Staphylococcus aureus* as a meat preservative. Although higher concentrations of MELE may be more effective in slowing down microbial growth in meat, these also reduce the consumer preference, and this may be a possible limitation to its application in the preservation of meat.

The synergistic combination of lower concentrations of MELE and 3% NaCl should be studied as a way of enhancing the stability of meat without adversely affecting the sensory attributes for possible application in food systems.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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