

The effect of *Moringa stenopetala* leaf extracts on microbiological contents and quality attributes of raw ground beef

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Abstract

Nowadays, health concerns associated with synthetic food preservatives draws the focus of researchers in the area more towards natural preservatives. In this study, the effects of *Moringa stenopetala* leaf extracts (MLE) on microbiological contents and other quality attributes of raw ground beef stored in low temperature condition were investigated. For this investigation, raw ground beef samples treated with different concentrations (0, 1.5, 2.5 and 5%) of MLE were used. The samples were stored at $4\pm 2^\circ\text{C}$ and experiments were conducted upto the 9th day of storage. The results indicated that the leaf extracts had significant ($P < 0.05$) effect on peroxide value, color, microbiological contents and sensory attributes, but had only small effect on pH value as compared to the control sample. Among the treated samples, samples treated with higher concentration of MLE maintained better overall quality and showed less microbial load than samples treated with less concentration of MLE. The study also indicated that sensory properties of the samples were affected by storage duration.

Keywords: Beef quality, raw ground beef, leaf extract, *Moringa stenopetala*, microbial growth

1. Introduction

Meat is the first-choice source of animal protein for many people all over the world [15]. Nutritionally, meat status is resulting from its high-quality protein, containing most essential amino acids, higher bioavailable minerals, vitamin B complex and fatty acids content [28]. However, after slaughter, meats including ground beef are easily susceptible to microbial spoilage, lipid oxidation, quality changes, and thus to economic loss [28]. Especially, recent studies have shown that the grinding of meat usually disrupts muscle cell membranes and expose the lipid membranes to metal ions which in turn act as pro-oxidants to initiate oxidation [18]. The oxidation process in ground meat affects the physical, organoleptic and nutritional properties of fresh meat by enhancing generation of rancid flavour and some oxidized compounds such as aldehydes, ketones and organic acids which are detrimental to consumer health [10].

The oxidation reaction is a complex process that repeatedly occurs in meat and meat products during processing and cold storage [19].

The speed of this reaction in meat depends on the degree of unsaturation of the fatty acids, the level of internal or external antioxidants and the existence of prooxidants, such as metal iron [19, 22].

Lipid oxidation and microbial growth are the main causes of food deterioration, poisoning, and wastage of meats. They strongly affects the safety, nutritious, sensory and economic potentials of stored minced meats [19, 22]. Indeed, limiting lipid oxidation and microbial growth in ground meat is a vital approach to maintain the safety, nutritional and sensory qualities, and economic potentials of meat products.

For controlling these undesirable changes in meat, synthetic preservatives have been widely used in the meat industry (Tang et al. 2001).

However, health problems associated with the consumption of meat treated with synthetic preservatives initiated researchers in the area to look for natural food preservatives.

The natural preservatives usually used in meats and meat products are plant extracts. Many researchers have reported that *Moringa stenopetala* plant extract contains high level of antioxidant, total phenolic, total flavonoid contents, and manifests antibacterial and antifungal activities [7, 26, 30]. Moringa leaf is rich essential amino acids, vitamins, beta-carotene, minerals, carbohydrates and low in fat [1,26].

Several researchers have also reported on the composition and preservative effect of *Moringa stenopetala* extracts. However, to our knowledge the effects of *Moringa stenopetala* leaf extracts on refrigerated ground beef quality and microbial growth are not yet investigated. Therefore, the main objective of this study was to investigate the effect of *Moringa stenopetala* leaf extract on microbiological contents and quality attributes of raw ground beef stored in low temperature condition.

2. Material and methods

2.1. Beef samples and chemicals used

Fresh boneless beef samples were purchased immediately after slaughter from local abattoir in Addis Ababa, Ethiopia. Then packed in icebox and transported to the Food Engineering laboratory of Addis Ababa Institute of Technology, Addis Ababa University. After removing connective tissues and fat; they were stored at $4\pm 2^\circ\text{C}$, approximately for 3 hours, until they were treated with *Moringa stenopetala* leaf extracts. *Moringa stenopetala* leaves were obtained from Araba Minch University's farm site, which is located about 327 miles from Addis Ababa, Ethiopia. They were also packed in icebox and transported to Food Engineering laboratory of the Addis Ababa Institute of Technology. Analytical grade chemicals such as glacial acetic acid, sodium thiosulfate, chloroform, potassium iodide, plate count agar, MacConkey agar, Mannitol salt agar, nutrient agar, peptone water were used for analyses

2.2 *Moringa stenopetala* leaf extraction method

Moringa stenopetala leaves were washed with distilled water until all the adhering foreign materials were removed and then dried in a room of average temperature 22.5°C until the moisture content was reduced to 7.5%. The dried leaves were ground into powder using grinder (Soni steel & Appliances, Mumbai, India) and sieved using 20 μm sieve size. 20 g of the *Moringa stenopetala* powder

was macerated in an enclosed flask of 180 mL solution containing 70 % ethanol and 30 % water for 24 hour in a dark room with constant shaking speed of 150 rpm as described by Peixoto et al. (2011) [25]. The macerated solution was centrifuged at 3000 rpm for 10 minutes followed by filtration through Whatmann no. 1 filter paper to separate the extract from the leaf residue. The extract was concentrated using vacuum rotary evaporator (RE-200A, Germany) to remove the solvent at 40°C . The extract was stored at 4°C until used.

2.3. Meat samples treatment process

Beef samples were ground in a meat grinder (NIMA.NM. -788 model:22, Germany). Then four, 480 g of raw ground beef samples were weighed, and each treated with different concentrations of *Moringa stenopetala* leaf extracts (0, 1.5, 2.5 and 5%). Control sample (0 % extract concentration) was prepared by mixing one of the 480 g ground beef samples in distilled water. The other samples were treated with different concentrations of the extract solutions. All the samples were stored in fridge whose temperature was kept at $4\pm 2^\circ\text{C}$ until used for analyses. Immediately after preparation, 120 g was taken from each sample and then analyzed for peroxide value, pH, color, microbiological and sensory analysis and reported as 0 day. The remaining samples were kept in the fridge until the next analysis days. The analyses were conducted on 3rd, 6th and 9th days of storage, and on each analysis day, 120 g was taken aseptically from each bag for the peroxide value, pH, color, microbiological content determination and sensory analysis.

2.4 Analysis of the samples

2.4.1. Determination of peroxide value

Peroxide value (PV) was determined according to the standard official method AOAC (2003) [4]. 50g of raw ground beef sample was macerated in 200 mL chloroform for 8 hours and filtered through Whatmann No.2 filter paper. 10 mL of the filtrate was collected in conical flask for evaporating chloroform using water bath.

After the removal of chloroform, the remaining filtrate was mixed with 30 mL chloroform and glacial acetic acid solution (2:1.5, v/v). Then, 0.5 mL of saturated potassium iodide solution (10%) added to the mixture and kept in the dark room for 5 minutes.

The iodine released during the reaction was titrated with a 0.01N sodium thiosulfate solution, and the peroxide value (PV) was expressed as milliequivalents of active oxygen per kilogram of the sample (meq O₂/kg of beef sample).

$$PV \left(\frac{\text{meq}}{\text{Kg}} \right) = \frac{(S-S_b) \times N}{W} \times 1000$$

where, PV: peroxide value, S: volume of titration (mL), S_b: volume of blank, N: normality of sodium thiosulfate solution (N = 0.01), and W: sample weight (kg).

2.4.2. Determination of pH

On each analysis day, 10 g of the meat sample was taken from each polyethylene bag aseptically and diluted with 100 mL deionized distilled water, and mixed for 60 s using a magnetic stirrer.

The mixture was filtered through a filter paper to obtain a clear filtrate for pH measurement. After calibrating at 4 and 7 pH using buffer solutions, digital pH meter (Model 3505, JENWAY) was used for the pH readings.

2.4.3. Color measurement

The color of raw ground beef was measured using Hunter lab Minolta spectrophotometer (CM -600d, JAPAN) with 20 mm aperture set for illumination D65 at 10° standard observer angles on each analysis day. The spectrophotometer was calibrated at the standard black and white color. The color coordinates CIE L* (lightness), a* (redness) and b* (yellowness) was measured perpendicular to the raw ground beef surface at three different points per samples. All the color parameters (L*, a* and b*) were obtained from the means of the readings.

2.4.4 Determination of microbiological contents

Microbial content in terms of Total Viable Count (TVC), Psychrotrophic count (PTC), Total coliform Count (TCC) and *Staphylococcus aureus* (*S. aureus*) were determined following the procedure of International Commission of Microbiological Specifications for Foods (ICMSF 1983). 10 g of each sample was taken aseptically and homogenized in 90 mL of sterile peptone- water solution (0.1 %). Serial 10-fold dilutions were made by pouring 1 mL of homogenate in 9 mL of the 0.1% peptone water solution, and then 0.1 mL of each diluted sample was inoculated onto plate count agar, nutrient agar, MacConkey agar and Mannitol Salt Agar medium to obtain the total viable count, Psychrotrophic count, total coliform count and *S. aureus* count,

respectively. The TVC, TCC and *S. aureus* plates were prepared in triplicate and incubated at 37°C for 48 hours. PTC plate was prepared in triplicate and incubated at 7°C for 7 days. The number of colonies was multiplied by the reciprocal of the respective dilution and expressed as log₁₀ of CFU/g of raw beef.

2.4.5. Sensory evaluation

A panel of 9 judges was used for the sensory analysis. All the panelists were postgraduate students in Food Engineering program of Addis Ababa University. The Panelists were asked to evaluate appearance, odour and overall acceptability of raw ground beef samples on 9-point hedonic scale: 1) dislike extremely; 2) dislike very much; 3) dislike moderately; 4) dislike slightly; 5) neither like nor dislike; 6) like slightly; 7) like moderately; 8) like very much; 9) like extremely [17]. All the samples were coded with 3-digit random codes and offered to the panelist in the random order. Samples were presented to the panelists just after opening the served samples to score appearance, odour and overall acceptability.

2.4.6. Statistical analysis

The statistical analysis was carried out using SPSS software package (IBM SPSS statistics version 24.0 for Windows; SPSS, Chicago, IL, USA). All the collected data were subjected to analysis of variance (ANOVA) and the mean separation was carried out using Tukey's test and statistical significance was checked at 95% confidence level (P < 0.05). The results were reported as mean ± standard deviation (SD).

3. Results and discussion

3.1. Effect of *Moringa stenopetala* leaf extract on peroxide values

Peroxide values (PV) of the raw ground beef samples treated with different concentrations of MLE are presented in Table 1. As shown in the table, on 0 day, there was no significant difference (P > 0.05) in the peroxide values of the samples. The peroxide values decreased significantly (P < 0.05) with concentration of the *Moringa stenopetala* leaf extract but increased with the storage duration. As can be observed from the table, the meat sample treated with 5% MLE had lower PV than the other samples analyzed on corresponding storage days. This could be linked to the presence of anti-oxidation substance in the *Moringa* leaves.

Several researchers in the area reported similar results. Falowo et al. (2017) [11], reported that *Moringa oleifera* leaf extract prevented lipid oxidation in raw ground beef. Other studies also indicated that *Moringa* leaf and seed extracts delayed lipid oxidation and thereby enhanced the shelf life of meat [3, 8, 23, 29], reported that addition of MLE at 0.1% retarded lipid oxidation of cooked goat meat patties stored at 4°C for 15 days which agrees with the findings of this work.

3.2. Effect of *Moringa stenopetala* leaf extract on pH values

The pH values of the raw ground beef samples treated with different concentrations of MLE are shown in Table 1. As shown in the table, the MLE had little influence ($P < 0.05$) on the pH of raw

ground beef during the first six storage days. This agrees with the finding of Muthukumar et al. (2014) [23], who reported the absence of variation in the pH values of control and MLE treated raw ground pork patty samples. During the first six storage days, the pH values were slightly increased from the values on the 0 day to the values on the 6 day. The slight increase in the pH of the sample during the storage period may be due to the fact that nitrogenous compounds such as ammonia are formed as a result of microbial activity [13] and deaminase reaction [31]. Decrements in pH values were observed after the 6th day for the samples treated with 2.5 and 5% MLE. This may be linked to the effect of moringa leaf extract either on microbial activity or on deaminase reaction or on both.

Table 1. Peroxide and pH values of raw ground beef samples stored at 4±2°C

Storage period (days)	MLE Concentrations			
	0%	1.5%	2.5%	5%
	Peroxide value (meq/kg)			
0	2.86±0.16 ^a	2.85±0.13 ^a	2.85±0.06 ^a	2.79±0.09 ^a
3	5.18±0.29 ^a	5.09±0.24 ^a	4.61±0.23 ^b	2.97±0.39 ^c
6	9.95±1.07 ^a	9.61±0.1 ^b	7.29±0.33 ^c	5.92±1.07 ^d
9	10.84±2.02 ^a	10.21±1.0 ^b	8.09±1.58 ^c	6.93±3.17 ^d
	pH value			
0	5.79±0.3 ^a	5.79±0.04 ^a	5.76±0.27 ^a	5.71±0.05 ^a
3	5.97±0.07 ^a	5.91±0.14 ^a	5.87±0.07 ^a	5.83±0.16 ^a
6	6.01±0.09 ^a	6.01±0.05 ^a	5.97±0.03 ^a	5.93±0.032 ^a
9	6.68±0.24 ^a	6.56±0.5 ^a	5.85±1.08 ^b	5.67±0.75 ^c

Means in the same row and columns followed by the same letter superscript are not significantly different ($P > 0.05$) and Values are mean ± SD of triplicate samples.

3.3. Effect of *Moringa stenopetala* leaf extracts on color values

The CIE color values of raw ground beef samples are illustrated in Fig. 1a. As shown in the figure, the lightness (L^*) values were slightly decreased ($P < 0.05$) as the concentration of MLE increased, with the lowest value (38.80±0.73) obtained for raw ground beef treated with 5% MLE on 0 day. However, the L^* values of all samples were gradually increased ($P < 0.05$) with the storage days. Similar results were reported for pork patties treated with MLE by Muthukumar et al. (2014) [23].

Similarly, Naveena et al. (2008) [24] also reported reduction in L^* values for chicken patties treated with pomegranate powder extract, and linked the cause for this reduction to the presence of pomegranate rind powder extract.

As expected, the addition of MLE increased the redness (a^*) values of raw ground beef as compared to the control (Fig. 1b). However, the a^* values of all the samples gradually decreased with the storage period. The color of meat changes during storage due to the oxidation of oxymyoglobin to met-myoglobin which results the formation of brown color that is commonly observed on the surface of raw meat [20]. As the progress of conversion proceeds from oxymyoglobin to met-myoglobin, a^* values start to decrease [12]. According to Muthukumar et al. (2014) [23] and Carpenter et al. (2007) [6], a^* values of ground pork patties showed progressive reduction during storage under refrigerated condition. Ahn et al. (2004) [2] also reported that the a^* value of raw ground beef increased with addition of natural extracts which agrees with the observations in this study.

The increase in redness values of raw ground beef could be linked to the presence of anthocyanins in natural plant extracts [21]. As can be seen from the slope of a^* lines, best color stability was observed on the sample treated with 5% MLE, during the entire storage period. This means that the color stability might be attributed to the presence of phenolic acids in the MLE. Similarly, Naveena (2008) [24], reported that the higher a^* values of chicken patties were due to the addition of pomegranate rind powder extracts, which is in close agreement with the findings in this work.

The yellowness (b^*) values of raw ground beef samples were significantly decreased ($P < 0.05$) with increasing in the concentration of the MLE as

compared to control (Fig. 1c). This might be due to the presence of coloring compounds in the moringa stenopetala leaf extract. However, as can be seen from the figure, b^* values are less sensitive at higher concentrations of MLE as compared to lower concentrations. The change in b^* values during storage duration is high for the control sample as compared to the treated ones which may be linked to the effect of the leave extract on post-mortem biochemical reactions contributing to color changes in meats. Several researchers reported the reduction in b^* of meats treated with different concentrations of leave extracts [23, 27].

Their findings are in close agreement with the results obtained in this study.

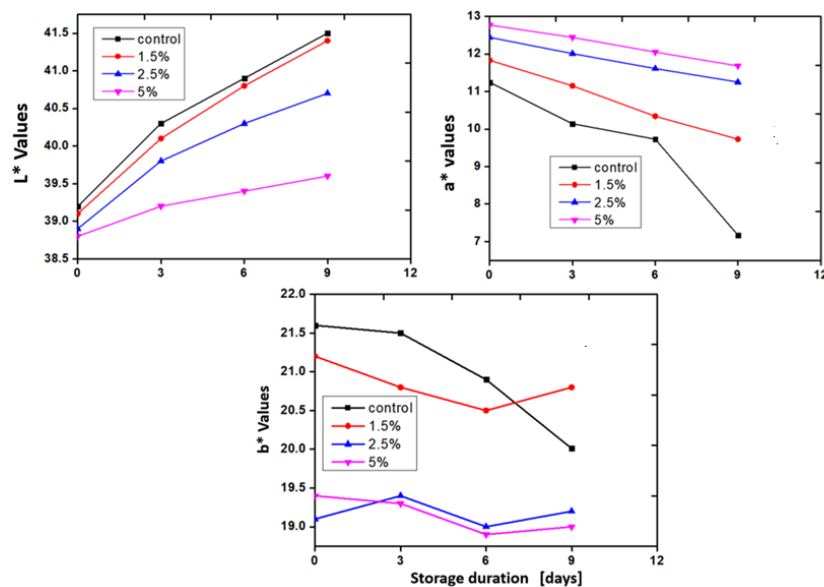


Figure 1. Depicts changes in (a) L^* values; (b) a^* values; (c) b^* values of raw ground beef during low temperature storage at different concentrations of MLE

3.4. Effect of *Moringa stenopetala* leaf extracts on microbiological contents

The effect of *Moringa* leaf extract on Total Viable Count (TVC), *S. aureus*, Total Coliform Count (TCC) and Psychrotrophic Count (PTC) of raw ground beef is illustrated in Fig. 2. Total Viable Counts of raw ground beef samples were evaluated and the means of the counts were presented as \log_{10} CFU/g in Fig. 2a. A total number of microorganisms exceeding $7.00 \log_{10}$ CFU/g of TVC is an indication of meat spoilage and potential health hazards as recommended by International Commission on Microbial Specification for Foods [16]. At the beginning of the storage period, no difference in TVC was observed among the treated samples. On the 3rd day, the control sample had

higher TVC ($4.92 \log_{10}$ CFU/g) than the samples treated with 1.5% ($4.65 \log_{10}$ CFU/g), 2.5% ($3.87 \log_{10}$ CFU/g) and 5% ($3.43 \log_{10}$ CFU/g) MLE. These values increased gradually during storage period, reaching $7.53 \log_{10}$ CFU/g for control sample, and 6.92, 4.14 and 4.06 \log_{10} CFU/g for 1.5, 2.5 and 5% MLE, respectively, on the 9th day. Other researchers also reported similar results: Falowo et al. (2017) [11] reported that in raw ground beef samples treated with moringa leaf extract lower TVC ($5.28 \pm 0.53 \log_{10}$ CFU/g) was observed as compared to the control sample ($5.80 \pm 1.05 \log_{10}$ CFU/g) after 3 days of storage at 4°C. Similarly, Hazra et al. (2012) [14], reported that the TVC of cooked buffalo meat was decreased significantly with the addition of moringa leaves

extract. Al-juhaimi et al. (2016), also reported that incorporation of moringa seed powder had significantly ($P < 0.05$) reduced the TVC of beef patties than control sample.

These variations in microbial growth rate among the samples might be linked to the differences in pH and antimicrobial levels, which could happen because of variations in MLE concentrations. The growth of *S. aureus* in the raw ground beef samples was significantly ($P < 0.05$) affected by the addition of MLE (Fig.2b). At the beginning of the storage period, no significant variation ($P > 0.05$) was observed in the *S. aureus* of the beef samples compare to the control. On 3rd and 6th days, the growth of *S. aureus* in the samples treated with 2.5% and 5% MLE decreased from 1.5 log₁₀ CFU/g to 1.44 and 1.23 log₁₀ CFU/g, respectively, but, increased to 2.76 and 2.12 log₁₀ CFU/g, respectively on the 9th day. Similarly, on 3rd and 6th days, the growth of *S. aureus* increased from 1.56 log₁₀ CFU/g to 2.84 and 2.55 log₁₀ CFU/g, for the control sample and the sample treated with 1.5% MLE, respectively. However, as shown in the figure, the growth of *s. aureus* in the control and 1.5% MLE treated samples was progressively increased during the storage period, and finally reached 3.42 log₁₀ CFU/g and 3.18 log₁₀ CFU/g on the 9th day, respectively. The maximum permissible level of *S. aureus* in meat is around 2.00 log₁₀ CFU/g as recommended by International Commission on Microbial Specification for Foods [16]. Therefore, the beef samples treated with 2.5 and 5% MLE remain below the maximum *S. aureus* acceptability limit until 6th days. Overall, the beef samples treated with 5% MLE had lower *s. aureus* growth than the other samples.

The moringa leaf extract also had significant effect ($P < 0.05$) on the growth of TCC (Fig. 2c). As shown in the figure, at the beginning of the storage period, the growth of the TCC in the treated and control beef samples is almost the same, and they showed steeper increase until the 3rd day. However, after this day, it started changing with concentration of MLE. The lowest TCC (1.96 log₁₀ CFU/g) was observed for the highest extract (5% MLE) concentration on 3rd day and the highest TCC (3.63 log₁₀ CFU/g) was measured for the control sample on 9th day. Overall, the growth of TCC increased significantly ($P < 0.05$) through the entire storage period for both the control and treated beef samples.

Differences in MLE concentrations brought variations ($P < 0.05$) on the growth of Psychrotrophic count (PTC) during the storage period (Fig. 2d). As shown in the figure, the magnitude of variation increased with storage days. From initial value of 3.61 log₁₀ CFU/g, PTC growth was observed increasing steadily during the storage duration and reached 7.34 log₁₀ CFU/g in the control sample on the 9th day. After the 3rd day, significant differences ($P < 0.05$) in PTC growth were observed among the samples, with higher values in control samples than the treated ones. In general, this finding revealed that the addition of MLE significantly ($P < 0.05$) reduced the growth of TVC, TCC, *S. aureus* and PTC in raw ground beef relative to control samples during the refrigerated storage duration. Similar result was also report by Baker et al. (2015) [5]. According to these authors, PTC growth on thyme-leaf extract treated lamb and chicken was substantially reduced when compared with the untreated ones.

3.5. Effect of *Moringa stenopetala* leaf extracts on sensory properties

The results in Fig. 3 show that the effect of MLE on sensory values (appearance, odor and overall acceptability) of raw ground beef during the storage duration. As shown in the figure, there was no significant variation ($P > 0.05$) in the sensory scores for the control and treated samples at the beginning of the storage time. The sensory scores for appearance, odor and overall acceptability of all treatments were significantly decreased ($P < 0.05$) as the storage duration increased. This might be due to degradation of protein and formation of off-flavor caused by lipid oxidation in the beef samples. The result of the sensory values of this work is in agreement with the findings of other researchers [9,17,32]. They reported significant reductions in the sensory attributes during low temperature storage of different meat products. As shown in the Fig. 3, the reduction of sensory attributes in control sample begins on the 3rd day, and rejection scores were observed after 6th days. However, the samples treated with 1.5% and 2.5% MLE revealed less scores ($P < 0.05$) with no negative impact on organoleptic characteristics during the 6th day, while poor organoleptic properties were observed on the 9th day.

The sample treated with 5% MLE showed the highest ($P < 0.05$) appearance, odor and overall acceptability values on 9th day.

Generally, starting from the 3rd day, appearance, odor and overall acceptability scores were significantly ($P < 0.05$) better in 5% MLE treated sample compare to the other samples. This shows that the use of 5% MLE improved the appearance, odor and overall acceptability of raw ground beef

compared to the other samples. This may be due to higher concentration of MLE is more effective in inhibiting peroxide effect or lipid oxidation than the less concentrations.

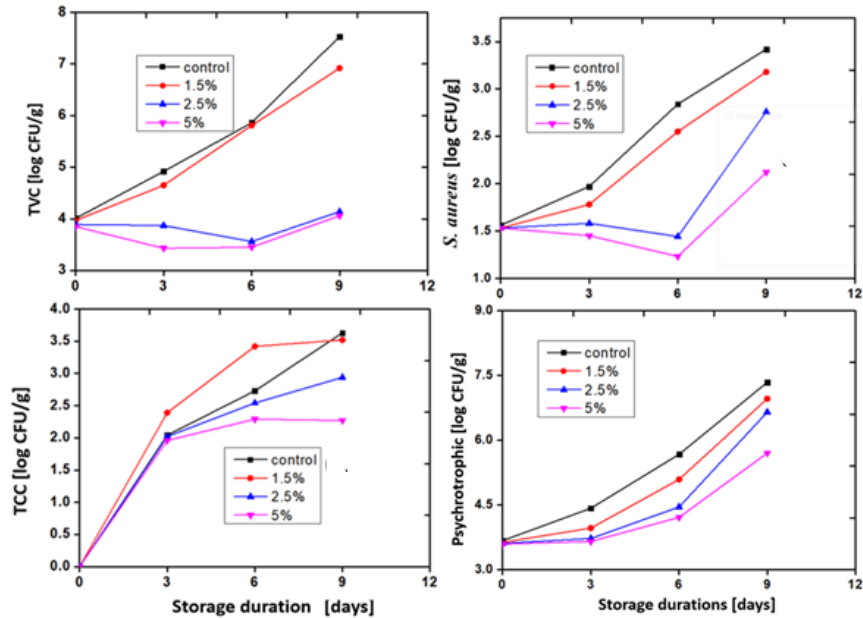


Figure 2. Depicts the effects of MLE on selected microbes at different concentrations (a) TVC (b) S. aureus (c) TCC (d) Psychotropic

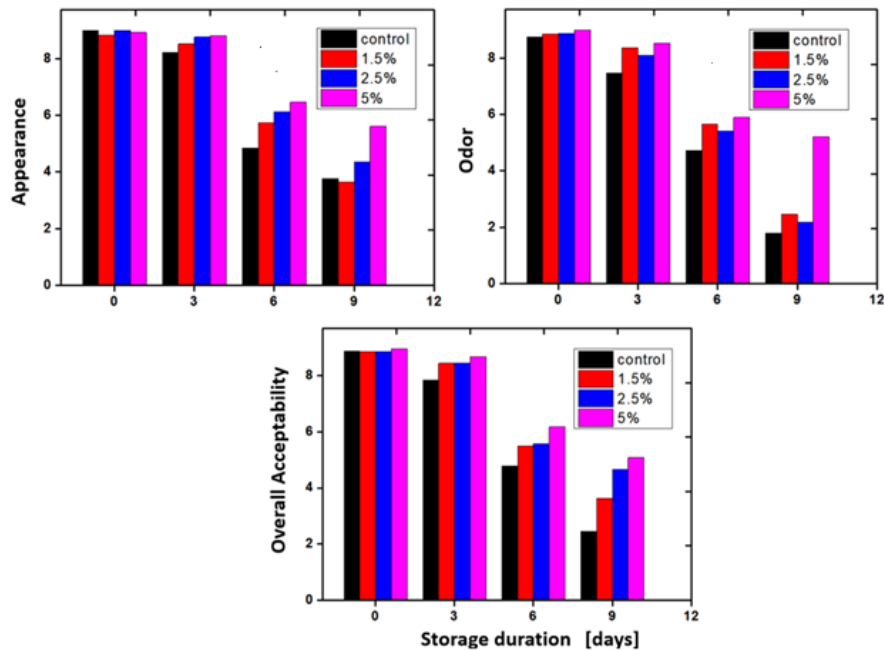


Figure 3. Depicts changes in (a) Appearance (b) Odor (c) Overall Acceptability of raw ground beef treated with different concentrations of MLE

4. Conclusions

The addition of *Moringa stenopetala* leaf extract to raw ground beef stored in low temperature condition generally resulted in positive consequence on the quality and microbiological contents of the beef samples.

The extract had significant effect on the peroxide value, Hunter lab color, microbial counts and sensory properties of raw ground beef, but had small effect on the pH values.

The effect appeared to be depended on the amount of extract used and hence, the beef sample treated with 5% MLE showed less microbial growth and maintained better fresh quality than samples treated with less/no MLE. The study also indicated that the use of *Moringa stenopetala* leaf extracts can extend the shelf life of raw ground beef stored at low temperature up to six days over that of the untreated beef. Therefore, these results indicate that MLE can be used as a natural antioxidant to maintain better quality and to retard microbial growth in raw ground beef during low temperature storage.

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Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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