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Effects of adding *Moringa oleifera* Leaves Powder on the Nutritional Properties, Lipid Oxidation and Microbial Growth in Ground Beef during Cold Storage

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Featured Application: The increase in health-conscious consumers and awareness in the prevention of diseases through diet opens a new market opportunity to improve meat products with functional ingredients such as *Moringa oleifera* leaves powder (MOLP). Meat products can be developed by enhancing their nutritional value either by decreasing fat content and/or incorporating new and functional ingredients such as MOLP. However, the meat industry encounters a serious challenge in maintaining traditional quality and reasonable cost of the formulated meat products. This study evaluated the influence of MOLP on the nutritional and technological properties, lipid oxidation, microbial growth, and sensory analysis of ground beef during cold storage. *Moringa oleifera* leaves powder improved the nutritional and technological properties and inhibited lipid oxidation in ground beef. Therefore, MOLP could be utilised by meat processors as a functional ingredient to enhance the quality attributes of ground beef.



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Abstract: The utilisation of *Moringa oleifera* leaves powder (MOLP) to improve the nutritional properties and inhibit lipid oxidation and the proliferation of microorganisms in ground beef during cold storage was examined. The effects of 0.2, 0.4, 0.6, and 0.8% MOLP on the nutritional properties (proximate composition, total phenolic and total flavonoid content), thiobarbituric acid reactive substances (TBARS), microbial composition, physicochemical characteristics (pH value, colour attributes, and cooking properties), and sensory analysis of ground beef were investigated. The findings showed that ash, protein, polyphenolic compounds, pH, colour, and microbial growth increased significantly, while moisture, fat content, and TBARS decreased significantly, with an increase in the concentration of MOLP during cold storage. Moderate levels (0.2 and 0.4%) of MOLP did not affect the sensory attributes of stored ground beef. Evidently, MOLP can be utilised as a natural preservative in ground beef to improve the nutritional value and inhibit lipid oxidation.

Keywords: *Moringa oleifera*; ground beef; lipid oxidation; microbial reduction; nutritional properties



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1. Introduction

Meat is a highly nutritious food rich in proteins, lipids, vitamins, and minerals. Nevertheless, chemical substances and microorganisms might reduce the nutritional quality of meat and its products [1]. These spoilage factors produce toxic substances in meat products that are hazards to consumers' health [2]. Spoilage of meat and meat products because of lipid oxidation and microbial multiplication have undesirable effects on the nutritional quality and result in tremendous economic losses [3]. Moreover, the activities of microorganisms might also have negative effects on the quality of meat due to the development of undesirable reactions that involve bad odour, colour, and textural changes in meat products [4]. Therefore, preservation of meat through antioxidants and antimicrobial agents plays an indispensable role, resulting in consumers buying safe and high-quality

meat and meat products. Meat preservation also inhibits lipid oxidation, activities of enzymes, and the proliferation of spoilage and foodborne microorganisms that result in economic and nutritional losses in the meat processing industry [5,6].

The utilisation of antioxidants is seen as a useful strategy in delaying or retarding lipid oxidation as well as inhibiting the generation of toxic oxidation products in meat products, thus enhancing the shelf-stability of food products [7,8]. At present, most consumers prefer processed meat products that are chemical preservative-free because of safety issues as well as health-related risks [9]. Moreover, utilisation of synthetic preservatives in meat products have proved ineffective in completely delaying spoilage microorganisms or preventing important foodborne pathogens such as *Listeria monocytogens* [10,11]. This explains the meat industry's renewed interest to search for natural antioxidants from plant extracts. The inclusion of natural antioxidants not only improves the shelf stability of meat products by delaying lipid oxidation because of polyphenolic compounds, but also enhances the physicochemical, textural, and organoleptic properties of meat products [12,13]. Therefore, the use of plant extracts that are rich in polyphenolic compounds enhances the quality and shelf life of meat products [14,15].

Moringa oleifera, which is customarily known as horse radish tree or drumstick tree, is regarded as a possible functional ingredient and auspicious source of natural antioxidants [16]. Almost all parts of *Moringa oleifera* (MO) plant such as roots, seeds, seed oils, leaves, and flowers have been utilised on a large-scale basis as food or components of medicine [17]. In addition, the leaves contain considerable amounts of antioxidant vitamin A, C, and E; protein; carotenoids; tocopherols; total phenols; and minerals such as iron, magnesium, potassium, and copper [18,19]. The leaves, therefore, have demonstrated their potential to be utilised as a functional ingredient in meat and its products such as ground meat and patties.

However, the use of MO as a non-synthetic preservative in ground meat is very limited. The powder of MO leaves might be a good choice in the production of healthier ground beef. This may also add value to the local production of the MO plant. Moreover, the nutrients in *Moringa oleifera* leaves powder (MOLP) could be conveniently concentrated because of the drying process, thereby improving the shelf life, handling, and storage of MOLP [20]. The presence of polyphenolic compounds in medicinal plants such as MO is associated with its preservative effect [21]. In light of this, the aim of this work was to produce ground beef fortified with MOLP and to determine the influence of its inclusion on the nutritional properties, lipid oxidation, and growth of microorganisms in the formulated ground beef.

2. Materials and Methods

2.1. Plant Material and Reagents

Moringa oleifera leaves were obtained from the University of Venda's experimental farm situated in Thohoyandou (22°58.08' S and 30°26.4' E and 595 msl), Limpopo Province, with minimum and maximum temperatures of 18 °C and 31 °C, respectively. The annual rainfall is approximately ± 500 mm. The leaves were collected in summer (October/November, 2019), and the moringa trees were planted in 2014. The reagents Folin-Ciocalteu, gallic acid, catechin, and thiobarbituric acid were obtained from Merck (Pty, Ltd., Midrand, South Africa). All reagents used were of analytical grade.

2.2. Preparation of *Moringa oleifera* Leaves Powder

Tap water was used to wash MO leaves to remove dirt and other foreign particles. The washed leaves were placed in a tray and exposed to the air to dry for approximately 20 min. Then, the leaves were dried again using an oven dryer (Prolab Model OTE 80.USA) at 50 °C for 3 h. The dried leaves were milled using Retsch miller (ultra-centrifugal mill ZM 200) and sieved using 40µm to obtain the final fine powder. Fine powder was put in a polyethylene bag, which was closed and stored in a cool dry place.

2.3. Preparation of Ground Beef

Four kilograms of boneless beef was purchased at a local super market in Thohoyandou, Limpopo Province, South Africa. Connective tissues and fat were removed through trimming and then the meat was cut into small cubes and minced using a meat grinder (P-22, Tallers Ramon, Barcelona, Spain) using 5 mm plates. Five formulations (control (F0) and treatments F1, F2, F3, and F4) of ground beef were prepared. The first formulation was used as a control (ground beef without MOLP), and MOLP was added at 0.2%, 0.4%, 0.6%, and 0.8% for formulations F1, F2, F3, and F4, respectively. The total weight of ground beef was 100 g and it was stored for 0, 5, 10, and 15 days in a refrigerator at 4 °C. The samples were packed in polyethylene plastic bags.

2.4. Proximate Composition

The Association of Official Analytical Chemists (AOAC) method [22] was followed to determine the moisture, protein, ash, and fat contents of ground beef. The moisture content of ground beef was determined according to the AOAC method 945.32 with oven drying at 105 °C for 3 h. Crude protein was determined using the Kjeldahl method and AOAC method 978.02, and $6.25 \times N$ factor was used. Ash content was determined using the muffle furnace according to the official method 923.03. The fat content was determined according to the AOAC method 920.39.

2.5. Polyphenolic Compounds

2.5.1. Total Phenolic Content

The Folin–Ciocalteu method was followed to measure the total phenolic content, as described by Mahmoud et al. [23]. Briefly, 2 g of each sample was weighed and transferred into beakers, and 20 mL of methanol acidified with hydrochloric acid (10%) was added and sonicated for 10 min in an ultrasonic bath, centrifuged (Rotina 380R-Labotec Ecotherm, Midrand, South Africa) for 10 min at $4000 \times g$, and then filtered. Afterwards, 0.5 mL of sample was transferred into test tubes and 1.5 mL of Folin–Ciocalteu reagent was added and allowed to rest at 25 ± 2 °C for 5 min. Then, 2 mL of sodium carbonate (7%) was added after another 5 min and incubated in a dark room for 45 min with an occasional shake. After incubation, the mixture developed a blue colour, and 10 mL of distilled water was added to dilute the colour. Finally, the absorbance of blue colour in different samples was measured at 725 using a UV spectrophotometer (Biowave II, 80-3003-75, Biochrom LTD, Cambridge, United Kingdom). Total phenolic content was expressed as milligram per gram gallic acid equivalent (GAE).

2.5.2. Total Flavonoids Content

A colorimetric method was followed to evaluate the total flavonoid content, as described by Ordonez et al. [24]. Briefly, a sample solution of 0.5 mL was added with an aliquot of 0.5 mL of 2% $AlCl_3$ ethanol solution. The samples were allowed to rest at 25 ± 2 °C for a period of 1 h. Afterwards, the absorbance of the sample was measured with a UV spectrophotometer (Biowave II, 80-3003-75, Biochrom LTD, Cambridge, United Kingdom) at 420 nm. A yellow colour showed the availability of flavonoids. Results were expressed as rutin (Ru) (mg/g).

2.6. Lipid Oxidation Measurement

Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS) throughout the storage period. The extraction method described by Witte et al. [25] was used to determine TBARS numbers (mg malonaldehyde/kg) with slight modifications. Two millilitres of ground beef extract was mixed with 2 mL of 0.1% thiobarbituric acid, and the mixture was centrifuged (Rotina 380R-Labotec Ecotherm, Midrand, South Africa) at $3000 \times g$ for 15 min. A boiling water bath at 95 °C for 60 min was used to heat the sample, and test tubes were allowed to cool at 25 ± 2 °C. A UV-visible spectrophotometer (Biowave

II, 80-3003-75, Biochrom LTD, Cambridge, UK) was used to measure the absorbance of the sample at 532 nm.

2.7. pH Analysis

The pH of ground beef was obtained by mixing 10 g of sample with 50 mL of distilled water using a stomacher (pbinternational, L7). The pH values were measured and recorded using a pH meter (Basic 2.0, Crison instrument, SA, Barcelona, Spain) equipped with penetration pH electrode. The electrode was calibrated or standardised against standard pH solutions of 4 and 8.

2.8. Microbial Analysis

The total plate count was enumerated on plate count agar (PCA, Merck, Sandton, South Africa), and Violet Red Bile agar (Merck, Sandton, South Africa) was used for total coliform count following incubation at 37 °C for 2 days. Yeast and mould were determined on potato dextrose agar after incubation at 25 °C for 5 days. Results were expressed as colony forming unit (CFU)/g [26].

2.9. Determination of Cooking Properties

2.9.1. Cooking Yield

The treated ground beef sample (10 g) was steamed for 1 min and allowed to cool at 25 ± 2 °C. Afterwards, a filter was used to surface-dry the cooked sample and it was weighed again using a weighing balance. The cooking yield was determined by considering the difference in weight of raw and cooked ground beef [27].

$$\text{Cooking yield} = \frac{\text{Weight of cooked ground beef}}{\text{Weight of raw ground beef}} \times 100$$

2.9.2. Fat Retention

The amount of cooking yield was multiplied with the amount of fat in the raw sample (unheated sample) to the amount of fat (heat treated sample) in order to calculate fat retention. The quantity of cooked food after heat treatment is the quantity of raw food [28].

$$\text{Fat retention} = \text{Cooking yield} \times \frac{\% \text{ Fat in cooked ground beef}}{\% \text{ Fat in raw ground beef}}$$

2.9.3. Moisture Retention

The moisture retention value represents the amount of moisture retained in the cooked ground beef per 100 g of raw ground beef sample, as described by El-magoli et al. [29].

$$\text{Moisture retention} = \frac{\text{percent yield} \times \text{percent moisture in cooked ground beef}}{100}$$

2.10. Colour Analysis

The colour was measured at three randomly chosen spots on the surface of the ground beef using a portable lovibond colorimeter (model no. LC 100, RM 200, Beijing, China), which was first calibrated using the black and white calibration tiles in order to measure the colour attributes L* (lightness), a* (redness), and b* (yellowness); from these coordinates, hue, chroma, and total colour difference were calculated using the equations below:

$$\text{Hue} = \tan^{-1} (b^*/a^*)$$

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

2.11. Sensory Evaluation

Seventy untrained panelists evaluated the samples on the fifth day of storage on the basis of the results of the microbiological analysis and for safety purposes. Ground beef samples were cooked in an electric oven for 30 min at 130 °C. All samples were given a code number and were kept warm for evaluation. Panellists were asked to evaluate the samples in terms of colour, texture, flavour, juiciness, and overall acceptability using a 9-point hedonic scale: 9 = extremely desirable and 1 = extremely undesirable. Tap water was given to the panellists to rinse their mouth after testing each sample.

2.12. Statistical Analysis

The analyses were performed in triplicate, and the mean data \pm SD (standard deviation) are reported. The Statistical Package for the Social Sciences (SPSS) software (version 23.0, IBM SPSS, Armonk, NY, USA) was used to analyse the data, and a two-way analysis of variance (ANOVA) was used with fixed effects of treatment, storage days, and their interaction. Control (F0) and treated samples (F1, F2, F3, and F4) and storage days (0, 5, 10, and 15) were used as factors. Differences between means were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Proximate Composition

Table 1 shows the proximate composition of ground beef and moisture content decreased as the concentration of MOLP increased, and there was a significant difference ($p \leq 0.05$) in the decreasing rates. The control samples had a higher moisture content compared to treated samples throughout the storage period. Serdaroglu [30] reported low moisture content of oat flour-treated beef patties due to an increase in total soluble solids. Therefore, high total soluble solids might have contributed to low moisture content of treated ground beef during cold storage days. Hawashin et al. [31] reported similar results, whereby the inclusion of destoned olive cake powder decreased the moisture content of raw beef patties.

The ash content of formulated samples significantly increased ($p < 0.05$) in comparison with the control with the increase of MOLP concentration from day 0 to day 15. The ash content increased within same sample as storage days increased, with this potentially being due to the fact that MOLP is very rich in minerals such as iron, calcium, potassium, and magnesium [32]. These findings are consistent with those of Alabi et al. [33], who found that ash content of Hubbard broiler chicken meat supplemented with a diet of aqueous MO leaf extract significantly improved.

The protein content of treated ground beef samples significantly increased ($p < 0.05$) with the increase of MOLP levels in comparison with the control sample. The protein content increased with the inclusion of MOLP within the same sample ranging from 20.09 for F0 sample in day zero to 22.15% in F4 sample in day 15. This suggests that MOLP is an excellent source of all essential amino acids, which are the building blocks of protein. The increase in protein content might have been due to antioxidant fractions found in moringa leaves [34]. A similar trend was reported by Subha et al. [35], whereby MOLP improved the protein content of rohu fillets in comparison with the control sample. Moreover, high protein content of treated ground beef might be attributed to the maturity stage of MO leaves, since young leaves tend to have a higher protein content than older leaves [36].

The findings of this study are the same as those of Aberra Melessea et al. [37], who demonstrated that the inclusion of different levels of *Moringa stenopetala* leaf powder improved the protein content of Arsi-Bale goat meat. Higher crude protein content of the MO products is beneficial to consumers, especially growing children who require protein in higher amounts.

Table 1. Effects of *Moringa oleifera* leaves powder (MOLP) on proximate composition (dry basis) of ground beef stored at 4 ± 1 °C for 15 days.

Parameters	Formulations (%)	Storage Time (Days)			
		0	5	10	15
Moisture (%)	F0	70.53 ± 0.49 ^{eI}	68.66 ± 0.29 ^{dG}	68.06 ± 0.06 ^{dF}	67.82 ± 0.09 ^{dF}
	F1	69.01 ± 0.80 ^{dH}	68.30 ± 0.06 ^{dG}	67.94 ± 0.06 ^{dF}	67.66 ± 0.07 ^{dE}
	F2	68.05 ± 0.19 ^{cF}	67.41 ± 0.08 ^{cD}	67.08 ± 0.07 ^{cD}	66.83 ± 0.09 ^{cD}
	F3	67.36 ± 0.15 ^{bD}	67.02 ± 0.06 ^{bD}	66.95 ± 0.07 ^{bD}	66.15 ± 0.10 ^{bC}
	F4	66.02 ± 0.11 ^{aC}	65.71 ± 0.30 ^{aD}	65.00 ± 0.02 ^{aB}	64.48 ± 0.19 ^{aA}
Ash (%)	F0	1.17 ± 0.02 ^{aA}	1.21 ± 0.02 ^{aB}	1.24 ± 0.01 ^{aB}	1.27 ± 0.01 ^{aC}
	F1	1.17 ± 0.04 ^{aA}	1.23 ± 0.04 ^{aB}	1.26 ± 0.04 ^{abC}	1.30 ± 0.02 ^{bD}
	F2	1.21 ± 0.03 ^{abB}	1.29 ± 0.02 ^{bC}	1.33 ± 0.02 ^{cD}	1.34 ± 0.03 ^{cD}
	F3	1.26 ± 0.01 ^{bC}	1.28 ± 0.03 ^{bC}	1.31 ± 0.03 ^{cD}	1.34 ± 0.01 ^{cD}
	F4	1.30 ± 0.05 ^{cD}	1.34 ± 0.01 ^{cD}	1.38 ± 0.02 ^{dE}	1.40 ± 0.01 ^{dE}
Fat (%)	F0	7.45 ± 0.07 ^{bJ}	7.42 ± 0.02 ^{dJ}	6.43 ± 0.13 ^{aF}	3.75 ± 0.09 ^{aA}
	F1	7.44 ± 0.05 ^{bJ}	7.35 ± 0.14 ^{bcI}	6.56 ± 0.11 ^{bG}	4.01 ± 0.08 ^{bB}
	F2	7.46 ± 0.16 ^{bJ}	7.32 ± 0.11 ^{bI}	6.70 ± 0.14 ^{cG}	4.47 ± 0.40 ^{cC}
	F3	7.02 ± 0.18 ^{aH}	6.91 ± 0.39 ^{aH}	6.71 ± 0.14 ^{cG}	4.76 ± 0.24 ^{dD}
	F4	7.62 ± 0.04 ^{cK}	7.30 ± 0.14 ^{bI}	6.75 ± 0.25 ^{cG}	4.97 ± 0.11 ^{eE}
Protein (%)	F0	20.09 ± 0.11 ^{aA}	20.18 ± 0.28 ^{aA}	20.56 ± 0.10 ^{bC}	21.12 ± 0.12 ^{aD}
	F1	20.14 ± 0.17 ^{aA}	20.44 ± 0.07 ^{bB}	20.72 ± 0.08 ^{aB}	21.43 ± 0.09 ^{bE}
	F2	20.58 ± 0.10 ^{bB}	20.75 ± 0.12 ^{cC}	20.96 ± 0.04 ^{cD}	21.60 ± 0.25 ^{cF}
	F3	20.87 ± 0.15 ^{cC}	21.31 ± 0.15 ^{dE}	21.53 ± 0.17 ^{dF}	22.00 ± 0.06 ^{dD}
	F4	21.13 ± 0.4 ^{dD}	21.54 ± 0.06 ^{eF}	21.96 ± 0.16 ^{eG}	22.15 ± 0.07 ^{dG}

Values expressed as mean ± standard deviation. Means with different small letters in the same column show significant difference among treatments within same storage day at $p \leq 0.05$. Means with different capital letters in the same column show significant difference among treatments across storage days at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

The fat content of treated samples decreased slightly but was still higher than those of the control from day 0 to day 15 within the same sample. This is because MOLP has a small amount of fat (2.3%) [38]. However, there was no significance difference ($p < 0.05$) in samples during days 0, 5, 10, and 15. Low fat content of treated ground beef samples ensures that beef maintains its quality. Too much fat undergoes oxidative degradation, resulting in rancidity, thereby decreasing the shelf stability of ground beef. Therefore, the control sample is more susceptible to spoilage than treated ground beef. The low fat content of treated ground beef samples may be due low fat content of MOLP [39]. Contrastingly, Nkukwana et al. [40] reported no significant difference in fat content of chicken breast meat supplemented with MO leaf meal.

3.2. Polyphenolic Compounds

The total phenolic content (TPC) of treated ground beef was significantly higher ($p < 0.05$) in comparison to the control from days 0, 5, 10, and 15 with increase in the concentration of MOLP, as shown in Table 2. This is associated with the level of TPC per unit volume of MOLP. Das et al. [41] indicated that the TPC of MOLP is 48.36 mg/g. The increase in TPC values of ground beef with increase in MOLP concentration is attributed to MOLP being a good source of antioxidants. The phenolic compounds found in medicinal plants such as moringa are of significant interest because they are associated with biochemical and pharmacological properties such as anticarcinogen and antioxidant effects [42]. Similar results were also reported by Negi and Jayaprakasha [43] and Naveena et al. [44], wherein the inclusion of pomegranate peels and pomegranate rind powder extracts increased the TPC of raw chicken patties.

Table 2. Effects of MOLP on polyphenolic compounds (dry basis) of ground beef stored at 4 ± 1 °C for 15 days.

Parameters	Formulation (%)	Storage Time (Days)			
		0	5	10	15
TPC (mg GAE/g)	F0	15.21 ± 0.08 ^{aB}	14.63 ± 0.4 ^{aA}	15.48 ± 0.50 ^{aB}	15.08 ± 0.05 ^{aB}
	F1	17.20 ± 0.10 ^{bC}	18.71 ± 0.31 ^{bE}	18.55 ± 0.24 ^{bD}	18.45 ± 0.05 ^{bD}
	F2	20.30 ± 0.05 ^{cF}	21.18 ± 0.12 ^{cG}	21.37 ± 0.05 ^{cG}	21.54 ± 0.07 ^{cH}
	F3	25.09 ± 0.09 ^{dI}	26.06 ± 0.07 ^{dJ}	27.17 ± 0.15 ^{dK}	27.43 ± 0.13 ^{dK}
	F4	32.27 ± 0.22 ^{eL}	32.75 ± 0.23 ^{eL}	33.05 ± 0.15 ^{eL}	33.33 ± 0.42 ^{eL}
TFC (mg CE/g)	F0	8.11 ± 0.48 ^{aA}	8.63 ± 0.30 ^{aB}	8.44 ± 0.14 ^{aB}	9.21 ± 0.20 ^{aC}
	F1	11.07 ± 0.09 ^{bD}	12.26 ± 0.14 ^{bE}	12.69 ± 0.28 ^{bF}	12.19 ± 0.03 ^{bE}
	F2	12.90 ± 0.41 ^{cG}	16.25 ± 0.19 ^{cH}	17.16 ± 0.05 ^{cI}	17.56 ± 0.08 ^{cI}
	F3	16.53 ± 0.08 ^{dH}	17.08 ± 0.31 ^{dJ}	18.66 ± 0.31 ^{dK}	19.45 ± 0.10 ^{dL}
	F4	19.28 ± 0.15 ^{eM}	19.60 ± 0.14 ^{eM}	21.12 ± 0.08 ^{eN}	21.84 ± 0.11 ^{eO}

Values expressed as mean ± standard deviation. Means with different small letters in the same column show significant difference among treatments within same storage day at $p \leq 0.05$. Means with different capital letters in the same column show significant difference among treatments across storage days at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

The total flavonoid content (TFC) of treated ground beef samples was significantly higher ($p < 0.05$) from days 0, 5, 10, and 15 within the same sample, with an increase in concentration of MOLP compared to the control sample. The TFC ranged from 8.11 in control sample (F0) during day zero to 21.84 mg/g for F4 sample in day 15, respectively. Mature MOLP is rich in flavonoid content [16]. Increase in TFC of treated ground beef shows the strong ability of MOLP to serve as a donor of hydrogen, reducing agents and singlet oxygen scavenger to inhibit lipid oxidation in meat [45]. Therefore, phenolic compounds as well as flavonoids probably enhanced the antioxidant activity of ground beef. Mahmoud et al. [23] reported similar results, whereby inclusion of orange peel improved the TFC of beef burger.

3.3. Lipid Oxidation and pH

Figure 1 shows the TBARS values of ground beef treated with MOLP during cold storage. The inclusion of MOLP decreased the TBARS values from days 0 to 15. The decrease of TBARS in treated ground beef might be due to polyphenols in MOLP, which adsorbs and neutralises free radicals, leading to the prevention of fat oxidation [46]. Fat oxidation and generation of volatile metabolites might be attributed to the increase in TBARS of ground beef during storage days [47,48]. However, samples treated with MOLP displayed delay in lipid oxidation at the end of storage day compared to the untreated sample (control).

Several studies have documented the positive relationship between reduced lipid oxidation and polyphenol content or antioxidant activity of plant extracts [1,49]. The association of natural substances such as polyunsaturated fatty acids with catalysts such as iron ion from the tissue of ground beef might have contributed to high values of TBARS in control sample throughout the storage days [50]. Such storage will ultimately promote the breakdown of heme compounds, thereby liberating the low-molecular-weight iron compounds in ground beef that are assumed to be accountable for lipid oxidation. Das et al. [51] reported similar results, wherein the inclusion of pre-blended carnosine decreased the TBARS values of ground buffalo meat during storage days. Moreover, the same authors reported that certain bacteria such as *Pseudomonas ovalis*, *Micrococcus freudenreichii*, as well as strains of *Streptomyces* also contribute to lipid oxidation by producing compounds such as aldehydes, ketones, peroxides, and carbonyls or other similar compounds.

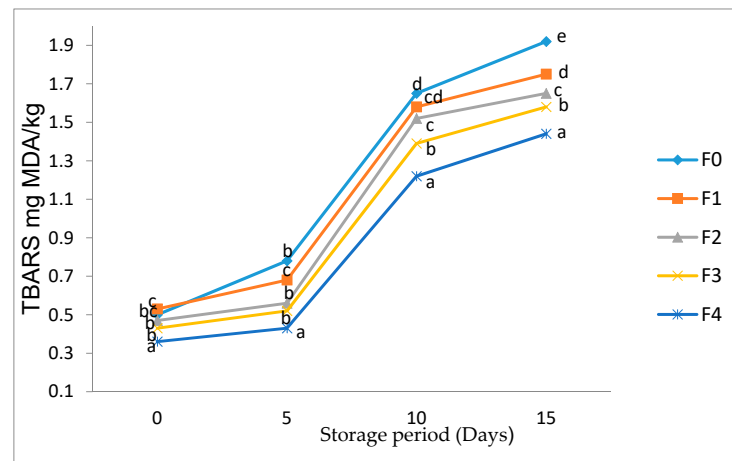


Figure 1. Effects of MOLP on thiobarbituric acid reactive substances (TBARS) of ground beef stored at 4 ± 1 °C for 15 days. Means with different superscripts show significant difference among treatments within same storage day at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

There were significant differences of pH values ($p < 0.05$) in all samples throughout storage days, except at day zero. Between days 0, 5, 10, and 15, the pH increased from 5.49 to 5.61, 5.74 to 5.44, 6.48 to 5.85, and 6.87–6.28, respectively, during storage days, as indicated in Figure 2. The high pH values of ground beef during storage might be attributed to the proliferation of Gram-negative bacteria such as *Pseudomonas*, *Moraxella*, and *Acinetobacter*, which resulted in the accumulation of metabolites [52]. Moreover, the rise of pH values throughout cold storage might have been due to bacteria utilising amino acids after the loss of stored glucose during the breakdown of proteins. This results in the build-up and generation of ammonia formation, which gives rise to the pH values of ground beef throughout the cold storage days [53].

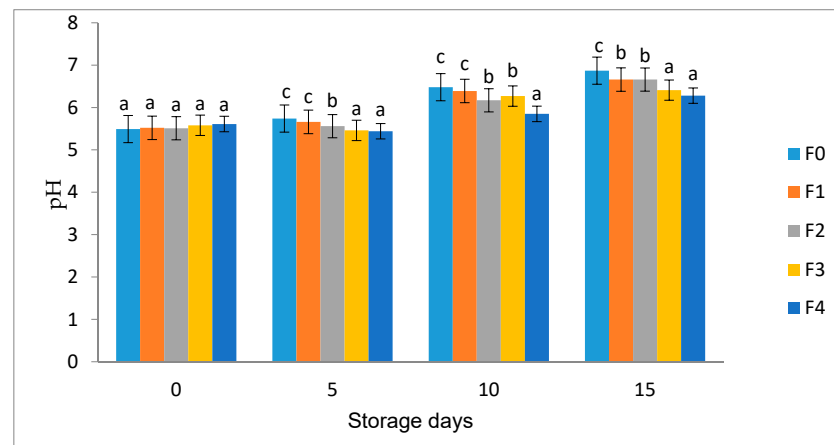


Figure 2. Effects of MOLP on pH of ground beef stored at 4 ± 1 °C for 15 days. Values are expressed as mean \pm standard deviation. Means with different superscripts show significant differences among treatments within same storage day at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

According to Wapi et al. [54], meat with a pH greater than normal of 5.8 is more prone to spoilage and results in lower shelf-life. Moreover, the higher meat pH results in less myoglobin, with the meat muscle becoming firm due to the high water holding capacity. Similar results were reported by Verma and Sahoo [55], wherein the incorporation of pre-blended tocopherol acetate increased the pH of ground chevon meat during refrigerated storage. Das et al. [56] also reported similar findings of a gradual rise in pH values of

ground and cooked chevon meat incorporated with curry leaf (*Murraya koenigii*) during the cold storage period of 20 days.

3.4. Microbiological Quality

The inclusion of MOLP did not affect the microbial properties of the ground beef during cold storage. The results show that the microbial population significantly increased during the storage days. Treated ground beef samples had lower microbiological counts at day 0 and increased with storage time from day 5 to 15, as indicated in Table 3. This could be attributed to the low dosage of MOLP, which was not enough to impart an antimicrobial effect on microbial growth. However, treated ground beef samples had lower microbial count during storage days in comparison with the control. The decrease in microbial count could be attributed to MOLP being an excellent source of phytochemicals such as flavonoids and phenolic acids, which are used as antimicrobials agents [57]. *Moringa oleifera* has a strong antimicrobial activity, with this being in line with the work of Okorundu [58], which notes that MO quantitative phytochemical screening showed that it contains alkaloids, flavonoids, cyanogenic glycosides, tannins, and saponins that can be successfully used to reduce and eventually destroy microbes in appropriate dosages.

Table 3. Effects of MOLP on microbiological quality in colony forming unit (CFU)/g of ground beef stored at 4 ± 1 °C for 15 days

Parameters	Formulations (%)	Storage Days			
		0	5	10	15
Total plate count	F0	5.18 ± 0.19 ^{eD}	10.49 ± 0.03 ^{eG}	21.59 ± 0.00 ^{eL}	31.47 ± 0.02 ^{eM}
	F1	3.85 ± 0.15 ^{dC}	8.42 ± 0.20 ^{dF}	17.47 ± 0.01 ^{dJ}	21.53 ± 0.01 ^{dL}
	F2	3.55 ± 0.06 ^{cC}	6.96 ± 0.01 ^{cE}	14.32 ± 0.01 ^{cI}	18.72 ± 0.01 ^{cK}
	F3	2.92 ± 0.07 ^{bB}	5.25 ± 0.05 ^{bD}	10.42 ± 0.01 ^{bG}	14.35 ± 0.12 ^{bI}
	F4	1.65 ± 0.03 ^{aA}	3.13 ± 0.01 ^{aB}	6.63 ± 0.01 ^{aE}	12.42 ± 0.39 ^{aH}
Total coliform	F0	4.55 ± 0.25 ^{eF}	7.36 ± 0.01 ^{eH}	16.24 ± 0.01 ^{eN}	29.95 ± 0.02 ^{eP}
	F1	3.17 ± 0.06 ^{dD}	5.33 ± 0.01 ^{dG}	12.59 ± 0.05 ^{dK}	16.83 ± 0.01 ^{dO}
	F2	2.56 ± 0.02 ^{cC}	3.94 ± 0.01 ^{cE}	10.09 ± 0.01 ^{cJ}	14.45 ± 0.07 ^{cM}
	F3	1.36 ± 0.01 ^{bB}	3.02 ± 0.01 ^{bD}	8.97 ± 0.00 ^{bI}	14.02 ± 0.01 ^{bL}
	F4	1.09 ± 0.01 ^{aA}	2.55 ± 0.02 ^{aC}	5.55 ± 0.02 ^{aG}	8.64 ± 0.01 ^{aI}
Yeast and mould	F0	5.56 ± 0.02 ^{eG}	7.14 ± 0.14 ^{eI}	17.75 ± 0.01 ^{eO}	19.65 ± 0.02 ^{eP}
	F1	4.97 ± 0.02 ^{dF}	5.01 ± 0.01 ^{dF}	11.61 ± 0.08 ^{dM}	15.80 ± 0.01 ^{dN}
	F2	4.02 ± 0.01 ^{cD}	4.45 ± 0.02 ^{cE}	6.60 ± 0.09 ^{bH}	11.06 ± 0.01 ^{cL}
	F3	2.45 ± 0.03 ^{bB}	2.94 ± 0.01 ^{bC}	7.52 ± 0.01 ^{cJ}	9.56 ± 0.02 ^{bK}
	F4	1.65 ± 0.02 ^{aA}	1.99 ± 0.00 ^{aA}	3.04 ± 0.01 ^{aC}	4.20 ± 0.07 ^{aD}

Values expressed as mean ± standard deviation. Means with different small letters in the same column show significant differences among treatments within same storage day at $p \leq 0.05$. Means with different capital letters in the same column show significant differences among treatments across storage days at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

Moreover, internal factors such as high protein and fat content, together with various external factors such as temperature and oxygen, which influence the behaviour of bacteria in food system as well as acting synergistically with preservatives such as antimicrobials, might also have contributed to the low microbial count in treated ground beef samples [59].

Neall [60] reported a wide spectrum of antimicrobial action in MO that works against most bacteria (Gram-positive and Gram-negative). Increasing MO level had a good influence because the antimicrobial mechanisms of phenol compounds rely on their level. Moreover, the coliform bacteria group, yeast, and mould could be reduced by decreasing free water. This is caused by the increased water binding ability of MOLP, which retards their growth [61]. The inclusion of MOLP in ground beef significantly ($p \leq 0.05$) decreased the coliforms in comparison with the control sample, demonstrating the protective role of MOLP, which could improve the safety of ground beef during cold storage. Nevertheless,

these results are in line with those reported by Muthukumar et al. [62] and Krishnan et al. [63]. They found that microbial growth increased during the storage period of ground pork patties and raw chicken meat treated with MOLP and spice extracts, respectively.

3.5. Colour Properties

During the storage days, the colour values of ground beef samples were influenced by the inclusion of MOLP in all treatments, as presented in Table 4. The lightness values of treated ground beef samples increased from days 0 to 15 with an increase of MOLP concentration. For the control sample, lightness values decreased with the passage of time. The low L^* value in the control sample might be attributed to the high levels of redness in meat and muscle pigment. Naveena et al. [45] reported a reduction in the L^* value of chicken patties with the inclusion of pomegranate peel powder extract. In addition, Rojas and Brewer [64] observed an increase in the L^* value of frozen vacuum-packaged pork and beef during the frozen storage period of 4 months, although it later stayed constant due to the inclusion of natural antioxidants. Moreover, the inclusion of MO seed flour increased the L^* values of beef patties [65].

The a^* (stability of the red colour) values significantly increased ($p < 0.05$) with the increase of MOLP concentration in treated ground beef samples. The control sample had lower a^* values throughout the storage period. Low a^* values of control sample during storage is related to oxidised myoglobin, metmyoglobin formation, and lipid oxidation of meat products [53]. Krishnan et al. [63] reported the probability of oxidation of pigment that catalyses lipid oxidation and produces free radicals that might oxidise the iron atom as well as denature the myoglobin molecules, causing a decrease in meat colour. Nevertheless, different factors can influence the stability of meat colour but the formation of metmyoglobin due to free radicals is the main cause of this phenomenon [66]. These results are similar to those of Liu et al. [67] and Kim et al. [50], wherein the inclusion of plant extracts improved the a^* values of beef patties.

There was reduction of b^* (yellowness) in the control sample in comparison with samples treated with MOLP during storage. The yellowness increased with an increase in concentration of MOLP but decreased with storage. The increase in yellowness of treated ground beef might have been due to the presence of carotenoids in MOLP [68]. However, these results are different from those of Shah et al. [53] and Muthukumar et al. (2014), who reported that the inclusion of MOLP decreased b^* values of beef patties and raw pork during storage.

The chroma significantly increased with the addition of MOLP in comparison with the control sample, but decreased with storage. The increase in chroma values of treated ground beef might have been due to an increase in a^* values with the addition of MOLP [69]. Therefore, the addition of MOLP improved colour intensity of ground beef. Nkukwana et al. [40] reported similar results, wherein the addition of MO leaf meal increased the chroma values of chicken breast meat.

The H^* (hue angle) value of the control sample was significantly ($p < 0.05$) higher than ground beef treated with MOLP. This implies that a high concentration of MOLP decreased the hue angle of raw ground beef. Therefore, MOLP shifted the hue angle to below the average in the control sample. These findings show a similar trend to that reported by Dzib et al. [70], wherein the inclusion MO meal decreased the H^* value of the Mexican hairless pig meat. Low a^* and C^* values and high H^* values indicate meat discolouration due to their positive association with concentration of metmyoglobin in meat and meat products [71].

Table 4. Effects of MOLP on colour properties of ground beef stored at 4 ± 1 °C for 15 days.

Parameters	Formulations (%)	Storage Days			
		0	5	10	15
L *	F0	42.90 ± 0.04 ^{aF}	42.17 ± 0.08 ^{aD}	41.34 ± 0.10 ^{aB}	40.75 ± 0.65 ^{aA}
	F1	43.01 ± 0.04 ^{aF}	42.68 ± 0.36 ^{bE}	41.69 ± 0.25 ^{bC}	41.33 ± 0.10 ^{bB}
	F2	43.26 ± 0.06 ^{bG}	42.99 ± 0.10 ^{bcF}	42.39 ± 0.05 ^{cE}	42.05 ± 0.13 ^{cD}
	F3	43.94 ± 0.07 ^{cH}	43.26 ± 0.07 ^{cG}	42.79 ± 0.16 ^{dE}	42.41 ± 0.24 ^{dE}
	F4	44.16 ± 0.08 ^{dH}	43.85 ± 0.07 ^{dH}	43.47 ± 0.07 ^{aG}	43.12 ± 0.13 ^{eF}
a *	F0	9.53 ± 0.21 ^{aE}	8.90 ± 0.91 ^{aC}	7.83 ± 0.13 ^{aB}	7.28 ± 0.12 ^{aA}
	F1	10.54 ± 0.07 ^{bG}	9.36 ± 0.06 ^{bE}	8.64 ± 0.26 ^{bC}	8.88 ± 0.11 ^{bC}
	F2	11.48 ± 0.49 ^{cH}	10.49 ± 0.13 ^{cG}	9.01 ± 0.06 ^{cD}	9.49 ± 0.52 ^{cE}
	F3	12.49 ± 0.30 ^{dK}	11.36 ± 0.0 ^{dH}	10.22 ± 0.08 ^{dF}	10.63 ± 0.16 ^{dG}
	F4	14.06 ± 0.15 ^{eL}	12.67 ± 0.07 ^{eK}	12.10 ± 0.04 ^{eJ}	11.77 ± 0.34 ^{eI}
b *	F0	15.20 ± 0.08 ^{aF}	14.14 ± 0.14 ^{aC}	14.00 ± 0.05 ^{aC}	12.87 ± 0.11 ^{aA}
	F1	16.13 ± 0.09 ^{bI}	15.75 ± 0.08 ^{bGH}	14.55 ± 0.39 ^{bD}	13.60 ± 0.04 ^{bB}
	F2	17.54 ± 0.04 ^{cL}	16.21 ± 0.28 ^{cI}	15.57 ± 0.33 ^{cF}	14.54 ± 0.08 ^{cD}
	F3	17.61 ± 0.06 ^{cL}	16.58 ± 0.29 ^{cJ}	16.12 ± 0.20 ^{dI}	14.92 ± 0.18 ^{dE}
	F4	18.35 ± 0.06 ^{dM}	17.83 ± 0.12 ^{dL}	16.98 ± 0.05 ^{eK}	15.94 ± 0.08 ^{eH}
Chroma	F0	17.94 ± 0.11 ^{aE}	16.73 ± 1.21 ^{aC}	16.05 ± 0.06 ^{aB}	14.79 ± 0.15 ^{aA}
	F1	19.27 ± 0.03 ^{bG}	18.32 ± 0.08 ^{bF}	16.92 ± 0.47 ^{bC}	16.24 ± 0.08 ^{bB}
	F2	21.02 ± 0.23 ^{cJ}	19.32 ± 0.31 ^{cG}	18.00 ± 0.61 ^{cE}	17.36 ± 0.30 ^{cD}
	F3	21.59 ± 0.18 ^{cK}	20.10 ± 0.26 ^{dI}	19.08 ± 0.21 ^{dG}	18.38 ± 0.24 ^{dF}
	F4	23.12 ± 0.87 ^{dL}	21.87 ± 0.07 ^{eK}	20.95 ± 0.03 ^{eJ}	19.82 ± 0.25 ^{eH}
Hue angle	F0	57.93 ± 0.61 ^{cG}	57.85 ± 2.84 ^{cG}	60.78 ± 0.46 ^{dJ}	60.50 ± 0.24 ^{cJ}
	F1	56.84 ± 0.34 ^{cF}	59.27 ± 0.20 ^{dH}	59.30 ± 0.10 ^{cH}	56.87 ± 0.29 ^{bF}
	F2	56.57 ± 1.19 ^{cF}	57.07 ± 0.13 ^{bc}	59.95 ± 0.53 ^{cdI}	54.89 ± 1.43 ^{bD}
	F3	54.71 ± 0.62 ^{bD}	55.57 ± 0.42 ^{abE}	57.59 ± 0.22 ^{bG}	54.29 ± 0.07 ^{abC}
	F4	52.49 ± 1.42 ^{aA}	54.60 ± 0.31 ^{aD}	54.52 ± 0.15 ^{dD}	53.55 ± 0.70 ^{aB}
ΔE	F0		2.59 ± 0.14 ^{dE}	5.53 ± 0.6 ^{dF}	12.00 ± 0.02 ^{dG}
	F1	1.39 ± 0.15 ^{bB}	1.92 ± 0.12 ^{cD}	1.36 ± 0.06 ^{aB}	1.92 ± 0.26 ^{cD}
	F2	1.82 ± 0.19 ^{cdD}	1.31 ± 0.13 ^{bB}	1.32 ± 0.06 ^{aB}	1.38 ± 0.11 ^{aB}
	F3	1.15 ± 0.12 ^{aA}	1.07 ± 0.30 ^{aA}	1.40 ± 0.07 ^{abB}	1.37 ± 0.55 ^{aB}
	F4	1.89 ± 0.47 ^{dD}	1.92 ± 0.25 ^c	2.18 ± 0.09 ^c	1.64 ± 0.10 ^{bC}

Values expressed as mean ± standard deviation. Means with different small letters in the same column show significant differences among treatments within same storage day at $p \leq 0.05$. Means with different capital letters in the same column show significant differences among treatments across storage days at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

The total colour difference (ΔE) of the control sample was significantly higher ($p < 0.05$) than ground beef samples treated with MOLP. However, there was no significant difference ($p > 0.05$) between treated ground beef samples during storage. The total colour difference is noticeable when it is beyond 2 [72]. On the basis of this information, the control sample had a measurable colour difference from 5, 10, and 15 days of storage, and the treated sample (0.8%) showed a noticeable difference at day 10. Other treated samples (0.2, 0.4, and 0.6%) did not show any measurable colour difference up to 15 days of storage, indicating that the addition of 0.2, 0.4, and 0.6% of MOLP does not change the colour of ground beef during storage. Nkukwana et al. [40] reported similar results, wherein the inclusion of MO leaf meal did not have significant effects on the total colour difference of chicken breast meat.

3.6. Cooking Properties

Table 5 shows the cooking properties of the control sample and treated ground beef. The inclusion of MOLP significantly ($p \leq 0.05$) influenced the cooking properties of ground beef. The cooking yield, moisture, and fat retention of treated ground beef significantly

($p \leq 0.05$) increased in comparison with control samples from day 0 to 15. The increase in cooking yield of treated ground beef might be attributed to MOLP's absorption of fat and water and its ability to maintain moisture in the matrix of ground beef [73]. In addition, the increase of ground beef pH due to the inclusion of MOLP likely accounts for the increase in cooking yield.

Table 5. Effects of MOLP on cooking properties of ground beef stored at 4 ± 1 °C for 15 days.

Cooking Properties	Formulations (%)	Storage Days			
		0	5	10	15
Cooking yield (%)	F0	51.56 ± 1.83 ^{aA}	52.58 ± 0.60 ^{aB}	53.95 ± 0.23 ^{aC}	56.03 ± 0.08 ^{aH}
	F1	52.63 ± 0.97 ^{abB}	54.17 ± 1.61 ^{bD}	54.24 ± 0.74 ^{aD}	56.23 ± 0.36 ^{aH}
	F2	53.65 ± 0.56 ^{bC}	55.16 ± 0.20 ^{bcE}	55.70 ± 0.61 ^{bF}	58.33 ± 0.25 ^{bJ}
	F3	54.30 ± 0.57 ^{bD}	55.71 ± 0.26 ^{cF}	56.51 ± 0.50 ^{bG}	59.12 ± 0.22 ^{cK}
	F4	57.61 ± 1.01 ^{cI}	58.27 ± 0.25 ^{dJ}	61.50 ± 0.59 ^{cL}	62.93 ± 0.35 ^{dL}
Moisture retention (%)	F0	46.11 ± 0.17 ^{aA}	47.83 ± 0.70 ^{aB}	47.85 ± 0.71 ^{aB}	49.41 ± 0.17 ^{aD}
	F1	46.39 ± 0.54 ^{aA}	49.14 ± 0.32 ^{bC}	50.75 ± 1.30 ^{bE}	51.15 ± 1.53 ^{bF}
	F2	49.00 ± 0.17 ^{bC}	54.41 ± 0.34 ^{cI}	53.41 ± 0.49 ^{cH}	56.98 ± 0.53 ^{cK}
	F3	52.32 ± 0.12 ^{cG}	55.88 ± 0.59 ^{dJ}	57.89 ± 0.66 ^{dL}	60.30 ± 0.20 ^{dM}
	F4	55.56 ± 0.24 ^{dJ}	57.57 ± 0.51 ^{eL}	61.28 ± 0.05 ^{eN}	61.91 ± 0.39 ^{eO}
Fat retention (%)	F0	58.98 ± 0.33 ^{aH}	57.49 ± 0.48 ^{bF}	54.38 ± 0.14 ^{aC}	52.56 ± 0.41 ^{bB}
	F1	59.13 ± 0.21 ^{aI}	56.42 ± 0.56 ^{aE}	55.80 ± 0.20 ^{bD}	51.50 ± 0.72 ^{aA}
	F2	61.27 ± 0.13 ^{bK}	58.35 ± 0.29 ^{cG}	56.52 ± 0.45 ^{bE}	52.64 ± 0.39 ^{bB}
	F3	62.60 ± 0.59 ^{cM}	61.74 ± 0.29 ^{dL}	57.74 ± 0.44 ^{cF}	54.51 ± 0.48 ^{cC}
	F4	64.64 ± 0.18 ^{dO}	63.05 ± 0.20 ^{eN}	60.36 ± 0.63 ^{dJ}	57.97 ± 0.74 ^{dF}

Values expressed as mean ± standard deviation. Means with different small letters in the same column show significant difference among treatments within same storage day at $p \leq 0.05$. Means with different capital letters in the same column show significant difference among treatments across storage days at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

Low cooking yield of the control sample during storage might be attributed to the reduction in the protein solubility as well as post-mortem enzymatic hydrolysis of ATP [51].

Improved moisture retention of treated ground beef samples could have been due to increased water absorption ability of protein powder and dissociation of proteins in the MOLP [74]. The inclusion of MOLP improved moisture and fat retention, with this potentially demonstrating the presence of a stronger structure of meat matrix in ground beef with the high concentrations of MOLP [75].

The increase in fat retention of treated ground beef samples could be attributed to swelling of starch and fibre. Moreover, the fat absorbed by the fibre might interconnect with protein matrix to prevent fat from migrating from ground beef [76]. Similarly, the fat and moisture retention of beef patties improved due to high water and oil binding capacity of MO meal flour [77]. Similar results were also recorded by Al-Juhaimi et al. (2016), wherein the inclusion of MO seed powder improved the cooking properties of beef patties.

3.7. Sensory Properties

Table 6 shows the effect of MOLP on the sensory analysis of raw ground beef stored at 4 ± 1 °C for 5 days and cooked in the oven for 30 min at 130 °C. Sensory properties such as colour, taste, springiness, and overall acceptability significantly decreased with the inclusion of MOLP, except for tenderness and juiciness. Similar results of decrease in sensory values of low-fat ground pork patties treated with carrageenan were reported by Kumar and Sharma [78] and were attributed to the decrease in moisture loss and surface dehydration during storage. The increase in moisture retention of the treated ground beef during cooking might be attributed to higher tenderness and juiciness. However, the control sample received a higher overall acceptability score, although there was no significant difference ($p > 0.05$) between samples F1, F2, and the control sample in all

sensory attributes. This is attributed to the inclusion of only a small amount of MOLP (0.2 and 0.4%).

Table 6. Effects of MOLP on sensory analysis of cooked ground beef stored at 4 ± 1 °C for 5 days.

Sample	Colour	Taste	Springiness	Tenderness	Juiciness	Overall Acceptability
F0	8.16 ± 0.66 ^c	7.90 ± 0.30 ^c	7.80 ± 0.08 ^b	7.80 ± 0.10 ^b	7.60 ± 0.02 ^b	7.50 ± 0.15 ^c
F1	8.10 ± 0.60 ^c	7.82 ± 0.25 ^c	7.75 ± 0.05 ^b	7.88 ± 0.09 ^b	7.65 ± 0.05 ^b	7.43 ± 0.11 ^c
F2	8.02 ± 0.55 ^c	7.78 ± 0.22 ^c	7.70 ± 0.06 ^b	7.90 ± 0.12 ^b	7.70 ± 0.08 ^b	7.40 ± 0.16 ^c
F3	7.50 ± 0.50 ^b	6.80 ± 0.08 ^b	6.50 ± 0.04 ^a	8.10 ± 0.15 ^a	7.90 ± 0.03 ^a	6.01 ± 0.10 ^b
F4	6.90 ± 0.45 ^a	6.30 ± 0.07 ^a	6.45 ± 0.03 ^a	8.20 ± 0.16 ^a	7.98 ± 0.04 ^a	5.60 ± 0.09 ^a

Values expressed as mean ± standard deviation. Means with different superscripts in the same column show significant difference among treatments at $p \leq 0.05$. F0 (100% ground beef), F1 (0.2%), F2 (0.4%), F3 (0.6%), and F4 (0.8%) *Moringa oleifera* leaves powder.

Low values of colour scores in treated ground beef may be associated with the green colour of MOLP, which arises from its chlorophyll content. The significantly lower taste acceptability of treated ground beef (F3 and F4) samples may be attributed to the bitter taste of MOLP. Bitterness in MOLP is due to the presence of the glucosinolate–myrosinase system, which is responsible for the bitter tastes in Brussels sprouts, kale, and collard greens [79]. Glucomoringrin and glucosoonjnain have been identified as the principal glucosinolates in MOLP, and therefore the activity and specificity of myrosinase is responsible for the bitterness [80]. The results suggest that the bitter taste is retained with the increase in MOLP concentrations. Moreover, inclusion of MOLP in ground beef resulted in unfamiliar odours. Our findings are similar to those reported by Jayawardana et al. [81], whereby consumers preferred the appearance, colour, odour, and taste of control sample chicken sausages added with 0.04% Butylated hydroxytoluene and 0.25% or 0.50 MOLP, with concentrations above 0.50% negatively affecting the sensory attributes.

4. Conclusions

The incorporation of MOLP enhanced nutritional properties such as polyphenolic compounds, protein, and ash contents of ground beef during cold storage. In addition, the inclusion of MOLP was effective in retarding lipid oxidation, with this improving the shelf stability of ground beef. Another advantage of incorporating MOLP in ground beef is the improvement of cooking properties. The results are due to the polyphenolic compounds, and antimicrobial and antioxidant characteristics of MOLP. However, the inclusion of MOLP did not affect the microbial quality of ground beef during storage. Our results demonstrate that MOLP can be utilised as a natural preservative in beef products. Up to 0.4% of MOLP can be used in ground beef without affecting sensory attributes.

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