Antioxidant and antimicrobial potentials of Damsissa (Ambrosia maritima) leaf powder extract added to minced beef during cold storage

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ABSTRACT
The antioxidant and antimicrobial effect of Damsissa leaf powder extract (DLPE) for application to cold-stored minced beef was investigated. DLPE exhibited inhibition zones of 14.0 and 12.7 mm against \textit{Escherichia coli} and \textit{Staphylococcus aureus}, respectively. Total polyphenol and flavonoid contents of DLPE were 188.06 mg gallic acid equivalent (GAE)/g and 2.56 mg catechin equivalent (CE)/g, respectively. DLPE exhibited a concentration-dependent increase \((P \leq 0.05)\) in total reducing power ability (TRPA), scavenging of \(\text{H}_2\text{O}_2\), and chelation of \(\text{Fe}^{2+}\) ions. Incorporation of different concentrations of DLPE in minced beef significantly \((P \leq 0.05)\) enhanced the physicochemical characteristics, sensory features, and microbial quality of the minced beef during cold storage \((4^\circ\text{C})\). DLPE \((2.5\%)\) was effective \((P \leq 0.05)\) in delaying lipid oxidation and preventing microbial growth in stored minced beef. Therefore, DLPE at 2.5\% concentration is recommended as a useful preservative to prolong the shelf-life of minced beef during cold storage.

1. Introduction
The recent decades witnessed a substantial increase in the worldwide production and consumption of minced beef. This has been owing to the population explosion, quick growth in the fast food market, and great nutritional value and palatability of beef meat products (Etillib, Elgasim, & Mohamed Ahmed, 2016; Hawashin, Al-Juhaimi, Mohamed Ahmed, Ghafoor, & Babiker, 2016). However, minced beef is susceptible to lipid oxidation and microbial spoilage because the mincing process disturbs muscles integrity, and with the increased surface area generated by mincing, facilitates the uptake of oxygen and microorganisms that leads to reduced shelf life (Honikel, 2014). Microbial activity and lipid oxidation adversely affect the safety, nutritional and organoleptic qualities, and economic value of meat products during storage (Hawashin et al., 2016; Jayawardana, Liyanage, Lalantha, Iddamalgoda, & Weththasinghe, 2015). Thus, retarding the microbial growth and lipid oxidation in minced meat by using natural or synthetic antioxidant and antimicrobial agents would be a useful strategy to reduce such harmful effects of increased lipid oxidation and microbial activity (Elhadi, Elgasim, & Mohamed Ahmed, 2017; Etillib et al., 2016). To date, several synthetic antioxidants and antimicrobials have been used to retard oxidative reactions, inhibit microbial growth, and consequently extend the shelf life of minced meat products (Fernandes, Trindade, Lorenzo, Munekata, & de Melo, 2016; Honikel, 2014). However, most of these synthetic preservatives are considered as unsafe ingredients by both consumers and health experts (Tang, Kerry, Sheehan, Buckley, & Morrissey, 2001) and thus their use in food preparations is regulated by several rules in recent years. Accordingly, interest in the use of natural...
Antimicrobials and antioxidants to prolong the shelf life of stored meat has markedly increased in last decades because of the expected health benefits and safety of these natural materials (Das, Rajkumar, Verma, & Swarup, 2012; Jayawardana et al., 2015). The use of natural antioxidants and antimicrobials in minced meat products is of high interest for both customers and meat producers to promote confidence in the safety of meat products and extend the marketing time of the products (Eltilib et al., 2016).

*Ambrosia maritima* L. ( Asteraceae) is a widely available weed in the Mediterranean region and African countries, particularly Egypt and Sudan, where it locally known as Damsissa and it grows abundantly near water catchments and on the banks of the Nile River (Saeed, Abdelgadir, Sugimoto, Khalid, & Effert, 2015). This weed plant is widely used in Sudanese traditional medicine for the treatment of urinary tract infections, gastrointestinal disturbance, kidney stones, diabetes, hypertension, asthma, rheumatic pain, and cancer (Dirar et al., 2014). It contains important sesquiterpene lactones, such as neoambrosin, ambrosin, and damsin, which have molluscidal and cytotoxic activities (Saeed et al., 2015). In addition, this plant contains several phytochemicals, such as coumarins, flavonoids, sterols and tannins, and exhibits considerable antioxidant activity (Dirar et al., 2014). Despite the common use of Damsissa in traditional medicine, to date, there are no reports on its use as a natural preservative of food products. Therefore, the primary aim of the current study was to investigate the antioxidant and antimicrobial potential of Damsissa leaf powder extract (DLPE) and to assess its influence on the physicochemical, microbial, and sensory qualities of minced beef.

2. Materials and methods

2.1. Preparation of Damsissa leaf powder extracts

Fresh Damsissa leaves were obtained from Toti Island (Khartoum, Sudan). The leaves were hand-cleaned to remove dust and any foreign material thoroughly washed with double-distilled water (ddH₂O), dried at ambient temperature, ground to a fine powder using an electrical mill, and stored in polyethylene bags at 4°C. Water extract was prepared by mixing 30 g of Damsissa leaf powder with 300 mL of distilled water and placed on an orbital shaker (Model OM 11, Ratek Instrument Ltd., Australia) at 150 rpm for 5 h at ambient temperature (25°C). After that, the extract was filtered using Whatman No. 1 filter paper and then centrifuged (HettichZentrifugen, Tuttingen, Germany) at 4000 × g for 10 min. The resultant supernatant was concentrated under reduced pressure at 40°C for 3 h using a rotary evaporator (IKA-WERKE-RV06ML, Stanfer, Germany). The concentrated extract was freeze-dried and stored in a freezer at −20°C until utilized. Prior to analysis of antimicrobial and antioxidant activities and the contents of total polyphenols and flavonoids, dried extract was reconstituted in ddH₂O at a final concentration of 30 mg/mL stock solution.

2.2. Preparation of minced meat

Fresh beef was obtained from a local meat market (Bahri, Khartoum North, Sudan). The meat was sliced, minced through a 0.75-inch plate using a meat mincer, and stored at 4 ± 1°C. Thereafter, DLPE at final concentrations (w/w) of 0%, 2.5%, and 5% were added to minced beef and then the content was thoroughly mixed. Instantly at the first day of preparation, minced beef samples from different treatments (0%, 2.5%, and 5% DLPE) were used for the analysis of the physicochemical, microbiological, and sensory attributes. The remaining samples were kept in a refrigerator (4 ± 1°C) for up to 10 days. At intervals of 5 days, minced beef samples were taken from each treatment and analyzed for physicochemical and microbiological properties.

2.3. Determination of chemical composition of Damsissa leaf powder and minced meat

The amounts of moisture, protein, fat, ash, and fiber in Damsissa leaf powder and minced beef were assessed as described in the standard method (AOAC, 2003). Carbohydrate was calculated by differences.

2.4. Determination of polyphenols and flavonoids of DLPE

Total polyphenols of DLPE was estimated as described by Price and Butler (1977) using Prussian blue spectrophotometric method. Gallic acid was used as external standard. Total polyphenol content was expressed as mg gallic acid equivalent (GAE) g⁻¹ sample. The flavonoids content was determined using a colorimetric assay as described by Zhishen, Mengcheng, and Jianning (1999). Briefly, 1 mL of DLPE was added to 0.3 mL sodium nitrite (5%, w/v) in a test tube, followed by 0.3 mL of 10% (w/v) aluminum chloride solution. The test tubes were incubated for 5 min at room temperature (25°C), and thereafter 2 mL of 1 M sodium hydroxide were added to the mixture. Instantly, the volume of the mixture was raised to 10 mL with ddH₂O,O, the mixture was vortexed, and the absorbance was determined at 510 nm. A calibration curve was prepared with catechin, and the results were presented as mg catechin equivalents (CE)/g.

2.5. Determination of antibacterial and antioxidant activities of DLPE

In the assay of antibacterial activity of DLPE, pure cultures of indicator microorganisms (*Escherichia coli* ATCC 29522 and *Staphylococcus aureus* ATCC 29218) were cultivated on agar plates and antibacterial activity was assessed using disc diffusion method as described by Kilani, Abdelwahed, Ammar, and Hyder (2005). In brief, DLPE saturated filter paper discs (10 µL extract/6 mm disc) were appropriately positioned on the surface of the cultured plates and the plates were incubated at 37°C for 24 h. After that, the plates were inspected for the occurrence of inhibition zones of bacterial growth around discs. Ampicillin (10 µg/disk) and chloramphenicol (30 µg/disk) were used as control standard antibiotics.

The antioxidant activities of DLPE were measured using three different antioxidant approaches, namely: total reducing power ability (TRPA), chelation of Fe²⁺ ions, and scavenging of hydrogen peroxide (H₂O₂) as described by Mohamed, Fageer, Eltayeb, and Mohamed Ahmed (2014). For TRPA assay, 1 mL of DLPE (125–1000 µg/mL) was mixed with sodium phosphate buffer (2.5 mL, 200 mM, pH 6.6) and potassium ferricyanide.
(2.5 mL, 1%). After 20 min incubation at 50°C, 2.5 mL of 10% trichloroacetic acid (TCA) was added, and then the reaction mixture was centrifuged at 2000 × g for 10 min. Thereafter, 2.5 mL of the supernatant was mixed with 2.5 mL of ddH₂O and 0.5 mL of freshly prepared FeCl₃ solution (0.1%), and then the absorbance was recorded at 700 nm against a blank. A higher absorbance indicates a higher reducing power. For the chelating activity of Fe²⁺ ions, 1 mL of DLPE (125–1000 µg/mL) was mixed with 1 mL of a solution containing 50 mM FeSO₄ and 50 mM NaCl (pH 7.0), and the mixture was incubated at room temperature for 20 min. Thereafter, 2 mL of 1 mM 2,2-dipyridyl was added, the absorbance of the complex of ferrous-dipyridyl was examined at 525 nm, and the results were expressed as a percentage of inhibition of 2,2-dipyridyl–Fe²⁺ complex formation (Harris & Livingstone, 1964). For H₂O₂ scavenging ability assay, 1 mL of DLPE (125–1000 µg/mL) in phosphate buffer (pH 7.4, 40 mM) was added to 1 mL of H₂O₂ solution (40 mM) in phosphate buffer (pH 7.4, 40 mM) and the reaction mixture was incubated for 10 min at room temperature. Then, the absorbance of H₂O₂ was determined at 230 nm against a blank solution containing phosphate buffer without H₂O₂, and the results were expressed as percent scavenging of H₂O₂ by the sample (Jayaprakasha, Rao, & Sakariah, 2004). In all assays, the spectrophotometric readings were carried out using a 1061-Shimadzu UV–VIS spectrophotometer (Shimadzu, Tokyo, Japan).

2.6. Determination of peroxide and pH values of minced beef treated with DLPE

The estimation of peroxide value was carried out following a standard official method (AOAC, 2003). Briefly, about 50 g of minced beef sample was soaked in 200 mL of chloroform for 8 h and then filtered through Whatman No. 4 filter paper. Thereafter, a saturated potassium iodide solution was added to a mixture of filtrate and chloroform/acetic acid (2:1.5, v/v) and kept in the dark. Then, released iodine was titrated with a sodium thiosulfate solution (0.1%), and then the absorbance was recorded at 700 nm against a blank solution containing 0.1% sodium thiosulfate and 0.1% 10% trichloroacetic acid (TCA) was added, and then the reaction mixture was centrifuged at 2000 × g for 10 min. The pH of the filtrate was measured using a digital pH meter (model 210, HANNA instruments microprocessor pH meter).

2.7. Microbiological analysis

The microbiological analysis methods described by Harrigan (1998) were used to determine the microbial quality of minced beef samples from different treatments and storage time. In brief, about 10 g of minced beef sample was homogenized with 90 mL of 0.1% sterile peptone water under aseptic conditions. Then, appropriate serial dilutions from each sample were prepared. Thereafter, 1 mL of each dilution was inoculated onto total plate count agar (PCA), MacConkey broth and Brilliant green bile lactose broth, MacConkey agar, and Baired-Parker broth (Oxoid, UK). After incubating the plates at 37°C for 24–48 h, they were respectively examined for total plate count, coliform, E. coli, and S. aureus counts.

2.8. Sensory analysis

Sensory analysis of prepared minced beef samples was carried out instantly after processing following standard guidelines for sensory evaluation (AMSA, 2015). A panel consisting of 20 members (10 male, age 26–40, and 10 female, age 24–33) of the staff and post-graduate students at the Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum provided sensory evaluation of cooked samples. Prior to the sensory evaluation, the panel received preliminary training sessions (n = 3) to focus attention on the sensory attributes so that each panelist was able to carefully discuss and identify each attribute. The samples with three-digit code numbers were served randomly to the panelists. Tap water was provided to the panel members between the samples to flush their palate from the previous sample taste. The panelists were requested to record their preference on a 7-point hedonic scale (7 = very acceptable, 1 = extremely unacceptable) for color, flavor, tenderness, juiciness, and overall acceptability of cooked minced meat products. Three independent sessions of sensory evaluations were performed, and the mean values of the scores of 20 panellists for each sample and session were calculated and used in data analysis. The cut-off score was set as 4 and the mean scores higher than 4 were considered acceptable.

2.9. Statistical analysis

Three minced beef batches were produced and all measurements were repeated three times. In each batch, three treatments (0.0% DLPE, 2.5% DLPE, and 5% DLPE) were performed and measurements (n = 3) were carried out at three storage days (0, 5, and 10). SAS program V. 8.1 (SAS Institute Inc., Cary, NC) was used for the statistical analysis of the results. The obtained data of physicochemical properties, microbiological, and sensory qualities from different treatments and storage times was subjected to analysis of variance (one-way ANOVA) using the general linear model (GLM) procedure. In the analysis models, the treatments, storage times, and assessors were considered as fixed factors, while replications of the experiments as a random factor. LSD was used to separate means and results were expressed as mean ± SD, and significances of the data were accepted at probability of P ≤ 0.05.

3. Results and discussion

3.1. Chemical composition of Damsissa leaf powder and extract

Table 1 shows the chemical composition of Damsissa leaf powder and extract. The results indicated that Damsissa leaf powder contains high amounts of carbohydrate (29.40 g/100 g), protein (26.20 g/100 g), and ash (20.67 g/100 g) and an appreciable amount of crude fiber (10.07 g/100 g), moisture (8.37 g/100 g), fat (5.30 g/100 g), whereas, DLPE contains substantial amounts of polyphenol (188.06 mg GAE/g) and flavonoids (2.56 mg CE/g). Strikingly, these results demonstrate that Damsissa leaves are a potentially excellent source of protein, carbohydrate, fiber, ash, polyphenols, and flavonoids. Thus, DLPE could be used as a source of these essential
nutrients in the diet to contribute to protein and energy requirements and provide the human body with bioactives that protect them from diseases arising from malnutrition and carcinogens (Salah & Yagi, 2011). The composition results show that Damsissa leaves contain nutritional and health potential that could find application as a food ingredient, in infant formula, food supplements, and food formulations. Several studies demonstrated wide range of bioactivities for Damsissa plant and its extracts, such as anthelmintic, antispasmodic, antidiabetic anti-inflammation, and antihypertension (Barakat, Al-Hizab, & Bakhiet, 2012; Makkawi, Keshk, ElShamy, & Abdel-Mogib, 2015) that are very relevant to many regions, such as Africa, South America, and Asia where the genus Ambrosia can be found. Dietary intake of up to 10% were found to be safe in animal models and exhibited significantly higher weight gain and significantly lower serum cholesterol and glucose levels in rats fed A. maritima compared to control treatment group (Barakat et al., 2012). A related plant, Ambrosia artemisiifolia L. was reported to have wide range of bioactive compounds that demonstrated a wide range of biological activities (Ding, Huang, Zhou, & Li, 2015). Collectively, these studies highlight the potential use of Ambrosia to provide functional role in food and pharmaceutical applications.

3.2. Antibacterial activity of DLPE

The in vitro antibacterial activity of DLPE against a Gram-negative (E. coli) and a Gram-positive (S. aureus) bacteria displayed inhibition zones of 15.0 mm (E. coli) and 12.7 mm (S. aureus), signifying high to moderate antimicrobial activities of DLPE against the two organisms (Table 2). Similarly, EL-Kamali and EL-amir (2010) reported that a methanol extract of A. maritima leaves had antibacterial activity against E. coli (15 mm, inhibition zone) and S. aureus (12 mm, inhibition zone). In addition, Wang, Kong, and Zhang (2006) reported similar observations on the antibacterial activity of leaf extracts of a closely related plant (Ambrosia trifida), grown in China, against E. coli and S. aureus. The inhibition potential of leaf extracts of A. maritima could be attributed to its high content of polyphenols and flavonoid compounds observed in the present study. In addition, leave extracts of A. maritima are reported to contain several bioactive compounds such as ambrosin, anhydrofranerisin, apigenin, s-amin, carvone, carophyllene, chloroambrosin, cineole, compoh, coumarins, damsin, dasmine acid, farnsiner, hymendin, hymenin, neoambrosin, stammon-b, sterols, β-sitosterol, tannins, and triterpenes with high antimicrobial and antioxidant activities (Badawy, Abdelgaleil, Suganuma, & Fuji, 2014; El-Sawy et al., 2017). These findings collectively demonstrate the protective potentials of leaf extracts of these plants, and thus, may pave the way for their utilization as a natural preservative in food products.

3.3. Antioxidant activity

DLPE exhibited substantial antioxidant activity as determined by three commonly used in vitro methods, such as TRPA, chelation of Fe²⁺ ions, and scavenging of H₂O₂ (Table 3). Previous reports have indicated that leaf extracts of A. maritima exhibited high antioxidant activity in the DPPH free radical scavenging assay (Dirar et al., 2014; Hilmi, Abushama, Abdalagdir, Khalid, & Khalid, 2014). Furthermore, Maksimovic (2008) found that leaf extracts of A. artemisiifolia L. expressed considerable DPPH radical scavenging and ferric-reducing antioxidant power activities. Our results show significant (P ≤ 0.05) increases in TRPA, Fe²⁺ ions chelation, and H₂O₂ scavenging activities with the increase in the concentration of DLPE from 125 to 1000 µg/mL. Previous reports have shown similar observations where TRPA, Fe²⁺ ions chelation, and H₂O₂ scavenging activities were increased with an increase in the concentration of leaf extracts of Moringa (Muthukumar et al., 2014; Shah, Bosco, & Mir, 2015) and Cyperus rotundus rhizome
extract (Eltilib et al., 2016). Our findings suggest that DLPE may act as an iron chelator, free radical and H₂O₂ scavenger and thus, it could prevent the formation of free radicals and the initiation of free radical-mediated chain reactions by stabilizing reactive species before they can participate in harmful reactions (Maksimovic, 2008). Due to its antioxidant potential, DLPE could be used in functional food formulations and therapeutic agents designed to prevent or slow the progress of aging and age-associated oxidative stress-related degenerative diseases.

3.4. Chemical composition of minced beef treated with DLPE

The chemical composition of minced beef was significantly (\(P \leq 0.05\)) affected by the addition of DLPE (Table 4) in a concentration-dependent manner. Increasing the concentration of DLPE in minced beef concurrently (\(P \leq 0.05\)) improved protein, fat, and ash contents, while the moisture content exhibited a concomitant (\(P \leq 0.05\)) decrease. A reverse association of moisture content and DLPE level may be correlated with the increase in the total solids, increased moisture binding ability or modification of the pH of the minced beef – DLPE system (Eltilib et al., 2016). Similarly, a concurrent reduction of moisture content following addition of increased concentrations of bambara groundnut seed flour and moringa seed and leaf powder to meat patties has been reported (Alakali, Irtwange, & Mzer, 2010; Al-Juhaime, Ghafoor, Hawashin, Alsawmahi, & Babiker, 2016; Elhadi et al., 2017). In addition, integration of bambara groundnut seed flour or moringa seed or leaf powder in meat patties simultaneously increased the protein, fat, and ash contents of the products (Alakali et al., 2010; Al-Juhaime et al., 2016; Elhadi et al., 2017). The increase in the crude protein, fat, and ash contents of minced beef supplemented with DLPE might be due to the high contents of these constituents in DLPE (Table 1). These findings established that integration of DLPE in minced meat could improve the nutritional value of the product.

3.5. pH and peroxide values of minced beef treated with DLPE during cold storage

Minced beef incorporated with increasing amounts of DLPE showed a significantly (\(P \leq 0.05\)) high pH values as the storage period progressed (Figure 1(a)). At all storage days, the pH of the control (0% DLPE) minced beef samples was lower (\(P \leq 0.05\)) than those incorporated with 2.5% and 5% DLPE indicating that DLPE enhances the pH of minced beef that could be due to the nature of bioactive compounds of DLPE that might not contain much acidic compounds. Generally, the reduction rate of pH was higher in control samples compared to DLPE-containing ones suggesting that DLPE enhances the pH stability of minced beef and could thus regulate the growth of spoilage microorganisms (Hawashin et al., 2016). Similarly, previous reports have shown that meat products formulated with moringa leaf and seed powder extracts exhibited higher pH values compared to unformulated controls (Al-Juhaime et al., 2016; Muthukumar, Naveena, Vaithiyathan, Sen, & Sureshkumar, 2014; Shah, Bosco, & Mir, 2015). Contrasting reports have indicated that incorporation of moringa leaf powder or extract in chicken sausages significantly reduced the pH of the product (Elhadi et al., 2017; Jayawardana et al., 2015). The divergent results obtained from these studies might be due to differences in materials and extracts used. Our findings also showed that over the storage period the pH of minced beef reduced significantly (\(P \leq 0.05\)). Similar observations on pH reduction during storage of sausages formulated with moringa leaf powder or extract (Elhadi et al., 2017; Jayawardana et al., 2015) and microencapsulated Jabuticaba (Myrciaria cauliflora) extract (Baldin et al., 2016) have been reported.

DLPE and storage period significantly influenced (\(P \leq 0.05\)) the peroxide value of minced beef (Figure 1(b)). Progress in storage period increased (\(P \leq 0.05\)) peroxide value of minced beef, whereas, increase of DLPE
concentration significantly ($P \leq 0.05$) decreased the peroxide value of the product. At the beginning of the storage period (0 time), minced beef samples with or without DLPE had similar ($P \geq 0.05$) peroxide values. The incorporation of DLPE in minced beef delayed ($P \leq 0.05$) lipid oxidation development because the peroxide values of DLPE containing samples were lower during storage than those of the control samples. This is in agreement with several reports that showed the incorporation of moringa leaf and seed extracts (Al-Juhaimi et al., 2016; Das et al., 2012; Shah et al., 2015) and microencapsulated Jabuticaba extract (Baldin et al., 2016) in meat products significantly reduced lipid peroxidation during cold storage. Reduction of peroxide values in the current study might be attributed to polyphenols and flavonoids of DLPE that possess antioxidant activity. Therefore, the incorporation of DLPE in minced beef inhibits the lipid oxidation and lower formation of $H_2O_2$, and it will thus contribute to extending the shelf life and the quality of the minced beef.

3.6. Microbiological characteristics of minced beef treated with DLPE

The microbiological analysis indicated that the counts of $E. coli$ and $S. aureus$ were less than $10^2$ cfu/g (data not shown), indicating that good hygienic conditions were followed during the minced beef processing. The microbiological characteristics of minced beef were different ($P \leq 0.05$) among all treatments and storage periods (Figure 2). The number of total plate and coliform counts of control samples were increased ($P \leq 0.05$) with the progress of the storage time. DLPE-containing samples exhibited significantly ($P \leq 0.05$) lower total plate and coliform counts throughout the storage period compared to untreated control. This showed that the DLPE treatment had significant ($P \leq 0.05$) antimicrobial activity and supported the aforementioned observations of the in vitro antimicrobial activity of the DLPE against $E. coli$ and $S. aureus$ (Table 1). Interestingly, the total plate counts of minced beef formulated with 2.5% and 5.0% DLPE were significantly reduced to 4.75 and 3.82 cfu/g, respectively after 10 days of storage. These values are considerably below the recommended detection limit (6.7 cfu/g) of the microbiological shelf life of minced beef (AFNOR, 2004). In contrast, the untreated control was significantly increased to 7.5 and 8.8 cfu/g after 5 and 10 days of storage, respectively. Our results show that the growth of bacteria in minced beef declined concurrently ($P \leq 0.05$) with the increase of DLPE level. Similarly, a reduction of total plate count in meat products incorporated with extracts of leaves and seeds of moringa have previously been reported (Al-Juhaimi et al., 2016; Jayawardana et al., 2015). By contrast, other reports indicated that inclusion of moringa leaf extracts, at relatively low doses, in meat products did not affect the microbial load of the products (Muthukumar et al., 2014; Shah et al., 2015). Overall, our findings demonstrate that incorporation of DLPE in minced beef substantially reduced the microbial load and thus extended the storability of the product for up to 10 days at 4°C.

3.7. Sensory attributes of cooked minced beef treated with DLPE

Inclusion of DLPE in minced beef reduced ($P \leq 0.05$) the panelist scores of the color, flavor, juiciness, tenderness, and overall acceptability compared to the untreated control (Table 5). The reduction trend in the scores of all sensory attributes was concomitant with increasing concentration of DLPE. Interestingly, all sensory attributes of minced beef incorporated with 2.5% DLPE had scores higher than 4.0 (cut-off score) indicating that minced beef treated with 2.5% DLPE was satisfactory and acceptable to the panelists. However, the flavor of 5% DLPE minced beef was acceptable and not different ($P \geq 0.05$) from the other treated samples. Jayawardana et al. (2015) stated that increasing the concentration of moringa leaf extract above 0.5% in chicken sausage

### Table 5. Sensory evaluation of minced beef treated with different concentrations of DLPE.

| Quality attribute | 0.0% | 2.5% | 5.0% |
|-------------------|------|------|------|
| Colour            | 6.08 ± 0.64<sup>a</sup> | 4.08 ± 0.19<sup>b</sup> | 3.54 ± 0.66<sup>b</sup> |
| Flavor            | 5.62 ± 0.33<sup>a</sup> | 4.46 ± 0.51<sup>b</sup> | 4.62 ± 0.45<sup>ab</sup> |
| Tenderness        | 6.00 ± 0.70<sup>a</sup> | 4.31 ± 0.03<sup>b</sup> | 3.39 ± 0.33<sup>b</sup> |
| Juiciness         | 6.15 ± 0.69<sup>a</sup> | 4.92 ± 0.95<sup>b</sup> | 3.15 ± 0.68<sup>b</sup> |
| Overall acceptability | 6.23 ± 0.93<sup>a</sup> | 4.39 ± 0.45<sup>b</sup> | 3.80 ± 0.80<sup>b</sup> |

Values are means of triplicate samples (±SE). *Means not sharing a common superscript in a row are significantly different at $P \leq 0.05$. Los valores son medias de muestras por triplicado (±SE). *Las medias que no comparten un superíndice común en una fila son significativamente diferentes en $P \leq 0.05$. 

![Figure 2](https://example.com/figure2.png)
negatively affected the sensory attributes compared to the control and those samples treated with 0.25% and 0.50% moringa leaf extract. In addition, other report has shown that increasing the concentrations of moringa seed powder in beef patties concurrently reduced the organoleptic attributes of the product (Al-Juhaimi et al., 2016). Nevertheless, inclusion of moringa leaf extract in meat burgers did not have any negative effect on the organoleptic characteristics of the product (Das et al., 2012; Muthukumar et al., 2014). The dissimilarities found in these reports could be due to variations in the parts of the moringa tree (seeds or leaves) that were used, the age of the trees, the percentage of the added plant materials, the meat type used, and the approaches used for addition of the extracts to the meat products. In the present study, the organoleptic attributes of cooked minced beef demonstrated that inclusion of up to 2.5% DLPE has resulted in very satisfactory sensory scores, albeit somewhat lower than those for non-treated controls, and thus could potentially be used in minced meat preservation.

4. Conclusion

This study provides, for the first time, evidence for the potential utilization of Damsissa leaf powder extract as a natural preservative for minced beef. Our study concludes that DLPE is an excellent source of flavonoids and polyphenols, and it contributes considerable antioxidant and antibacterial activity to minced beef during refrigerated storage. The incorporation of DLPE in minced beef significantly enhanced its physicochemical characteristics, oxidative and storage stability, and safety. While the majority of the sensory attribute scoring of the minced beef product were lower with addition of DLPE, 2.5% DLPE appeared to be well within the range of acceptability for consumers. Therefore, utilization of DLPE to prolong the shelf life and the safety of minced beef products could both satisfy the expectation of consumers for natural, healthy and safe food ingredients and add value to this underutilized Damsissa weed plant.

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