Antioxidant activities of *Moringa oleifera* L. and *Bidens pilosa* L. leaf extracts and their effects on oxidative stability of ground raw beef during refrigeration storage

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**ABSTRACT**

The study examined the leaf extracts of *Moringa oleifera* and *Bidens pilosa* for presence of bioactive phytochemicals and their antioxidant activities on pH and lipid oxidation of fresh ground beef during 6 days cold storage. The results revealed that *B. pilosa* leaf extract contained higher amount of bioactive compounds and antioxidant contents \((p < 0.05)\) than *M. oleifera* leaf extract. The extract of *B. pilosa* leaf exhibited higher antiradical activity against 2,2-Diphenyl-2-picrylhydrazyl and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radicals than *M. oleifera* leaf extract and standard butylated hydroxytoluene (BHT) \((p < 0.05)\). Addition of *M. oleifera* and *B. pilosa* leaf extracts \((0.5 \text{ and } 1 \text{ g/kg})\) to fresh ground beef were found to lower the pH and thiobarbituric acid-reactive substances values compared with control and BHT treatments \((0.2 \text{ g/kg})\) during the storage period \((p > 0.05)\). The antioxidant activities of the extracts indicate that *M. oleifera* and *B. pilosa* leaf can be used as nutraceuticals or preservative agents in food industry.

**INTRODUCTION**

Application of plant extract in food industry as natural antioxidant and preservative agents has continued to receive a considerable attention in recent times due to their ability to prolong shelf life and enhance consumer health. As natural antioxidant, plant extract can donate hydrogen ions to inhibit free radical formation and/or interrupt propagation of autoxidation in muscle food (Falowo, Fayemi, & Muchenje, 2014). As potential preservative agents, plant extract possesses huge bioactive compounds which are capable of disrupting and degrading the cytoplasmic membrane and cell wall of spoilage microorganisms (Kim, Cho, & Han, 2013; Radha Krishnan et al., 2014) and also improve the physicochemical qualities of processed meat products (Shah, Don Bosco, & Mir, 2015; Velasco & Williams, 2011).

Presently, processed meats represent a large percentage of muscle foods consumed in the Western world (Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015) because they are easily accessible and relatively inexpensive compared with traditional fresh meat cuts (de Oliveiraa et al., 2012). However, due to production process, almost all processed meats including ground or minced beef are easily susceptible to lipid and pigment oxidation. Recent studies have shown that the grinding of meat usually disrupt the muscle cell membranes and expose the lipid membranes to metal ions which in turn act as pro-oxidants to initiate oxidation (Kim et al., 2013). The initiation of oxidation process in ground meat limits their shelf life and compromises the physical and nutritional quality of meat by generating rancid flavour and oxidized compounds (aldehydes, ketones and organic acids) which are detrimental to consumer health (Falowo et al., 2014).

To deal with these undesirable changes and reduce the use of synthetic preservatives, extracts from plant sources...
are added to meat and meat products as natural additives (Falowo et al., 2014; Velasco & Williams, 2011). Interestingly, extracts from Moringaceae (Moringa oleifera Lam.) and Asteraceae (Bidens pilosa Linn.) plant families are known to contain rich antioxidant compounds (Adedapo, Jimoh, & Afolayan, 2011; Moyo, Masika, Hugo, & Muchenje, 2012). The leaves of these plants have been used for centuries as dietary ingredients or supplements (Bartolome, Villaseñor, & Yang, 2013; Hazra, Biswas, Bhattacharyya, Das, & Khan, 2012). Recent studies on their application have shown that they possess great biological activities such as anti-diabetes, anti-tumor, anti-inflammation, antinfective and antibacterial (Bartolome et al., 2013; Dai & Mumper, 2010). Reports on their nutritional contents have also showed that they are rich in proteins (including essential amino acids), vitamins, beta-carotene, minerals and low in fat and carbohydrates (Adedapo et al., 2011; Moyo et al., 2012; Bartolome et al., 2013). The antioxidant and biological activities of these plants have been attributed to the presence of phytochemicals including flavonoids and other phenolics in their leaves extract (Al-Owaisi, Al-Hadiwi, & Khan, 2014; Falowo, Muchenje, Hugo, & Charimba, 2016).

Despite the above-mentioned qualities, limited studies are available on the efficacy of M. oleifera extracts (Hazra et al., 2012; Muthukumar, Naveena, Vaithiyathan, Sen, & Sureshkumar, 2014; Shah et al., 2015) and to our knowledge, the preservative effect of extract from the leaves of B. pilosa in meat products as potential antioxidants has not been studied. Therefore, the objective of this study was to investigate the effect of M. oleifera and B. pilosa leaf extracts on the oxidative stability of ground meat from cattle. Prior to application of the extracts in meat samples, the phytochemical constituents and antioxidant activities of the plant leaves were also determined.

Materials and methods

Chemicals

Gallic acid, 2,2-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-diazinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4'-disulfonic acid, sodium carbonate, butylated hydroxytoluene (BHT) and rutin were purchased from Sigma Chemical Co. (St. Louis, MO, USA), n-hexane, Aluminium chloride (AlCl₃), Folin–Ciocalteu phenol reagent and sodium carbonate were from Merck (Damstadt, Germany). All other chemicals used including the solvents, were of analytical grade.

Plant sample and extract preparation

Bidens pilosa and M. oleifera leaf were obtained from the University of Fort Hare farm (South Africa) and Moringa South Africa Ltd, respectively. The M. oleifera was grown commercially by Moringa South Africa, and processed by air-drying while B. pilosa was processed by oven-drying at 40°C for 12 hr, and milled into powder through a 2 mm sieve. The dry plant samples (200 g) were exhaustively macerated with 800 ml of ethanol-water solution (7:3) at room temperature for 2 days. Each extract was separated from the residue by filtration using Whatmann no.1 filter paper and then concentrated under reduced pressure at 55 °C using a rotary evaporator. The extracts were lyophilized with a freeze-drier and the dried extracts were used for the determination of the antioxidant activity at concentration of 1 mg/ml. Determination of the nutritive values of the plants were carried out on the dry samples. All analyses were done in triplicate. The dried powder of plant extracts were then stored at 20°C for further analysis. The phenolic contents of the extracts have been analyzed previously (Falowo et al., 2016).

GC-MS analysis of the crude extracts

The free radical scavenging activity of extracts on DPPH radical was estimated using the method described by Liyana-Pathiranan, Shahidi, and Alasalvar (2006). A solution of 0.135 mM DPPH in ethanol was prepared and 1.0 ml of this radical solution was mixed with 1.0 ml of sample solution. The reaction mixture was incubated in the dark for 30 min at room temperature and then the absorbance was measured at 517 nm using spectrophotometer. Rutin and BHT were used as reference standards. The ability of the extract to scavenge DPPH radical was calculated by the following equation:

\[ \text{DPPH radical scavenging activity (β) = } \frac{[\text{Abs control} \times \text{Abs sample}]}{[\text{Abs control}]} \times 100 \]

where Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + sample extract /standard. The DPPH radical scavenging activity (%) of the extracts was analyzed in triplicate.
ABTS radical scavenging activities

ABTS radical cation decolourization assay to determine the free radical scavenging activity of plant extracts was carried out as described by Re et al. (1999). Stock solutions (ABTS\textsuperscript{+}) were prepared by reacting a 7 mM ABTS solution with 2.4 mM potassium persulphate solution in equal quantities and the mixture was allowed to stand in the dark at room temperature for 16–18 h before use. The stock solution was then diluted by mixing 1 ml ABTS solution with 53 ml of ethanol to obtain an absorbance of 0.705 units at 734 nm. One millimeter of diluted ABTS working standard solution was mixed with 1 ml of plant extract/standard and the absorbance was measured after 7 min at 734 nm using the spectrophotometer. The ABTS scavenging capacity of the extracts was compared with that of rutin and BHT as reference standards. The percentage inhibition was calculated as ABTS radical scavenging activity (%) = ([Abs control – Abs sample])/[Abs control] × 100, where Abs control is the absorbance of ABTS radical + ethanol; Abs sample is the absorbance of ABTS radical + sample extract/standard. The ABTS scavenging capacity of the extracts was analyzed in triplicate.

Preparation of beef samples

Fresh beef samples (Muscularis longissimus thoracis et lumbrorum) were obtained from 40 nondescript cattle at high throughput commercial abattoir and processed after 48 h postmortem. Beef samples were cut into small cubes after removal of visible fat and connective tissues and minced in a sterile meat grinder (CombinMax600, China). A portion (1200 g) of the ground beef were randomly assigned to one of the following treatments: (1) NC (negative control, meat without additives); (2) BP (meat with 1 g/kg B. pilosa extract); (3) ML (meat with 1 g/kg M. oleifera extract), (4) BPML (meat with 1 g/kg B. pilosa and M. oleifera extract) and (5) PC (positive control, meat with 0.2 g/kg BHT). Immediately after adding the extracts and BHT, the ground beef were aerobically packed in polyethylene bags (O2 permeability = 6000–8000 cm\textsuperscript{2}/(24 h × m\textsuperscript{2} × atm), water vapor transmission = 83 g/(24 h × m\textsuperscript{2}) and 50% relative humidity) and stored at 4 ± 1°C and analyzed on 0, 3 and 6 days of storage for pH and thiobarbituric acid-reactive substances (TBARS).

PH determination

The pH of the fresh ground beef sample was determined as described by Muthukumar et al. (2014) with slight modifications. A 5 g portion of the sample was blended in 25 ml of deionized distilled water for 60 s using homogenizer (Model Polytron\textsuperscript{®} PT 2500 E Stand Dispersion Device, Kinematica AG, Switzerland). The pH values were measured using a standardized electrode attached to a digital pH meter (CRISON Instruments S.A., Alella, Spain). The pH analysis was carried out in eight replicates per each treatment and storage day.

Determination of lipid oxidation

The lipid oxidation of the fresh ground beef was determined by quantifying the TBARS in 5 g of sample using the aqueous acid extraction method of Raharjo, Sofos, and Schmidt (1992). The values of TBARS obtained were multiplied by 10 and expressed as micrograms of malonaldehyde (MDA) per gram of meat. All TBARS analysis was carried out in four replicates per each treatment and storage day.

Statistical analysis

Data obtained on antioxidant contents of the plant extracts were analyzed using PROC ANOVA procedures of the Statistical Analysis System (SAS, version 9.1.3 of 2007). The pH and lipid oxidation values were analyzed using PROC GLM procedures of SAS (version 9.1.3 of 2007). Differences in mean values were computed using Tukey’s Studentized Range procedures for multiple comparisons.

Results and discussion

Identification and quantification of phytochemicals of moringa oleifera and biden pilosa leaf extracts

The phytochemical composition of the extracts as revealed by GC-MS analysis is presented on Table 1. The extract of B. pilosa exhibited more volatile compounds (20 compounds) than M. oleifera (13 compounds) during maximum run time of 18.23 min (Figures 1 and 2). The number of compounds identified in this study was relatively higher than those reported by Al-Owaisi et al. (2014) for M. oleifera extract and Chien et al. (2009) for B. pilosa extract. This difference could be due to variation in extraction solvents and method of analysis. The use of different solvents has been reported to cause noticeable effect and greater differences in chemical composition of extracts (Mohamed, Ali, El-Baz, Hegazy, & Kord, 2014). Among the phytochemicals identified in the extracts (Table 2), the most prevailing compounds which have been reported for strong antioxidants activities were tetradeconoic acid (Mujeeb, Baijpai, & Pathak, 2014), n-hexadecanoic acid, and hexadecanoic acid ethyl ester (Rajeswari, Murugan, & Mohan, 2012), phytol (de Moraes et al., 2014), DL-alpha-tocopherol (Di Mambroa, Azzolinib, Valimb, & Fonseca, 2003) and phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)]-4-methyl (Mujeeb et al., 2014). Antioxidant compounds are the major constituents of medicinal plant and they possess redox properties that can adsorb and neutralize free radicals, quench singlet and triplet oxygen or decompose peroxide in cell and muscle food (Adedapo et al., 2011; Moyo, Oyedemi, Masika, & Muchenje, 2012). Other biological activities which have been reported for the identified compounds include antimicrobial, anticancer, anti-inflammatory, anti-diabetic, hypocholesterolemic and cell death prevention (Cisneros, Paredes, Arana, & Cisneros-Zevallos, 2014; Peng et al., 2015; Yin et al., 2014).

Antioxidant activities of the plant extracts

The antioxidant activities of plant extracts are presented in Table 3. The extracts of M. oleifera revealed higher amount of antioxidant activities than the extract of B. pilosa. The percentage inhibition of DPPH radicals (p < 0.05) for M. oleifera extracts, B. pilosa extract, rutin, and BHT were 75.9, 77.1, 73.8 and 70.6% while that of ABTS radicals were 82.8, 83.24, 79.3 and 85.0%, respectively (Table 3). The DPPH radical scavenging activities of B. pilosa and M. oleifera extract demonstrated significant strong antioxidant activity, and compared favourably with the standard rutin and BHT which are
Table 1. Chemical composition of leaf extract of *Moringa oleifera* and *Bidens pilosa*.

| No | Compounds                                                                 | Retention time (min) | % Peak area |
|----|----------------------------------------------------------------------------|----------------------|-------------|
| 1  | Tetradecanoic acid                                                         | 10.677               | 7.54        |
| 2  | n-Hexadecanoic acid                                                        | 10.734               | 2.23        |
| 3  | Hexadecanoic acid, ethyl ester                                             | 10.827               | 20.59       |
| 4  | Phytol                                                                    | 11.425               | 6.05        |
| 5  | 9,12-Octadecadienoic acid (Z,Z)                                            | 11.581               | 6.44        |
| 6  | Linoeleic acid ethyl ester                                                 | 11.650               | 8.39        |
| 7  | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)                        | 11.696               | 36.36       |
| 8  | Octadecanoic acid, ethyl ester                                             | 11.756               | 3.91        |
| 9  | 1,5-Cyclooctadiene                                                         | 12.650               | 2.77        |
| 10 | Heptasiloxane, 1,1,3,3,5,5,5,7,7,9,9,11,11,13,13-tetradecamethyl           | 12.832               | 0.42        |
| 11 | 4-Dehydroxy-N-(4,5-methylenedioxy 2-nitrobenzylidine) tyramine             | 12.867               | 1.40        |
| 12 | DL-alpha-Tocopherol                                                        | 16.305               | 2.67        |
| 13 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl         | 18.113               | 1.24        |

*Bidens pilosa*

| 1  | Bicyclo(2.2.1)heptan-2-one, 1,7,7-trimethyl, (1S)                          | 5.760                | 0.94        |
| 2  | Benzamide, 4-methoxy-N-[2-(1-methylcyclopropyl) phenyl                     | 8.844                | 0.71        |
| 3  | 9 H-Fluorene, 9-diazo                                                     | 9.601                | 11.34       |
| 4  | Hexadecanoic acid, ethyl ester                                            | 10.812               | 15.98       |
| 5  | Phytol                                                                    | 11.415               | 5.58        |
| 6  | Benzo[h]quinoline, 2,4-dimethyl                                           | 11.547               | 1.14        |
| 7  | Linoeleic acid ethyl ester                                                 | 11.632               | 3.64        |
| 8  | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-                       | 11.667               | 8.80        |
| 9  | S-Acetamido-4,7-dioxo-4,7-dihydro benzo furan                             | 12.022               | 5.60        |
| 10 | Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane                    | 12.139               | 0.29        |
| 11 | 1 H-indole-2-carboxylic acid, 6-(α-ethoxyphényl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester | 12.352               | 0.51        |
| 12 | 1,2-Bis (trimethylsilyl) benzene                                          | 12.530               | 0.23        |
| 13 | 5-Methyl-2-trimethylsil oxy-acetophenone                                  | 12.662               | 1.02        |
| 14 | Phenol, 2,2'-methylenebis(6-(1,1-dimethylethyl)4-methyl                   | 12.835               | 7.67        |
| 15 | Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl        | 13.053, 13.547, 18.113 | 1.01 |
| 16 | Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl            | 13.113, 13.479       | 0.64        |
| 17 | Tris (tert-butyl dimethyl siloxy) arsane                                   | 13.171, 13.853       | 0.95        |
| 18 | Cyclotrisiloxane, hexamethyl                                              | 14.646               | 1.65        |
| 19 | 4-Methyl-2-trimethylsilyloxy-acetophenone                                 | 17.561               | 2.51        |
| 20 | Arsenous acid, tris (trimethylsilyl) ester                                 | 18.091               | 1.39        |
derivatives of phenolic compounds. In overall, the ABTS radical scavenging activities of extract showed greater antioxidant activity than DPPH radical. This could be attributed to differences in mechanism of action and reaction of DPPH and ABST radical. The ABTS has been reported to be soluble in aqueous and organic solvents, and can therefore determine both hydrophilic and lipophilic antioxidant capacities (Abegg, Alabarse, Schuller, & Benfato, 2012). However, our observations agreed with the finding of Moyo et al. (2012) who found that scavenging ability of M. oleifera extract against ABTS was greater than DPPH radicals. The radical scavenging activities of M. oleifera extract observed in this study were comparable with those reported by Sultana, Anwar, and Ashraf (2009) while that of B. pilosa extract were slightly lower than those reported by Adedapo et al. (2011). Also, the presence of chlorophyll and carotenoid has been reported to contribute significantly to antioxidant activity of plant species through their ability to scavenging reactive oxygen species, singlet molecular oxygen and peroxyl radicals (Bunea et al., 2012). The chlorophyll a (2.62 ± 0.05 mg/g DW) and b (0.98 ± 0.01 mg/g DW) contents in B. pilosa were significantly higher (p < 0.05) than those of M. oleifera leaves (Table 4). The total chlorophyll values were 3.60 ± 0.04 and 1.46 ± 0.01 mg/g DW for B. pilosa and M. oleifera respectively (p < 0.05). The total carotenoid content was higher for B. pilosa (0.73 ± 0.00 mg/g DW) and lower for M. oleifera (0.39 ± 0.00 mg/g DW) (Table 4). The presence of total chlorophyll and carotenoid together with synergistic effect of phytochemicals could be responsible for stronger free radical scavenging activities displayed by B. pilosa extract in this study.

Effect of moringa oleifera and bidens pilosa leaf extracts on pH

The pH value on fresh ground beef during storage at 4°C is shown in Table 5. There was no significant difference (p > 0.05) in the pH values of the fresh ground beef across
Table 2. Bioactivity of phytocomponents identified in the leaf extracts of *Moringa oleifera* (M. oleifera) and *Bidens pilosa* (B. pilosa) by GC-MS.

| Compound | Extract | Compound Structure | Molecular Weight | Activity                |
|----------|---------|--------------------|------------------|------------------------|
| 1        | Tetradecanoic acid | *M. oleifera* | C_{14}H_{30}O_{2} | 228                    | Antimicrobial          |
| 2        | n-Hexadecanoic acid | *M. oleifera* | C_{16}H_{32}O_{2} | 256                    | Antioxidant            |
| 3        | Hexadecanoic acid, ethyl ester | *M. oleifera* | C_{16}H_{32}O_{2} | 284                    | Antimicrobial          |
| 4        | Phytol | *B. pilosa* | C_{20}H_{40}O | 296                    | Antimicrobial          |
| 5        | Octadecan-18-one, 1, 3, 5, 7, 9, 11, 13, 15 -hexadecamethyl | *B. pilosa* | C_{16}H_{32}O_{2} | 578                    | Antimicrobial          |
| 6        | Linoleic acid ethyl ester | *M. oleifera* | C_{20}H_{40}O_{2} | 308                    | Antimicrobial          |
| 7        | DL-alpha-Tocopheryl | *M. oleifera* | C_{20}H_{40}O_{2} | 430                    | Antioxidant            |
| 8        | 9, 12-Octadecadienoic acid (Z,Z) | *M. oleifera* | C_{18}H_{32}O_{2} | 280                    | Antimicrobial          |
| 9        | Phenol, 2',2'-methylenebis [6-(1,1-dimethylethyl)]-4-methyl | *B. pilosa* | C_{3}H_{6}O | 220                    | Antioxidant            |
| 10       | 4-Methyl-2-trimethylsilyloxy-acetophenone | *B. pilosa* | C_{3}H_{6}O_{2} | 306                    | Antimicrobial          |
| 11       | 9,12,15-Octadecatrienonic acid, ethyl ester, (Z,Z) | *B. pilosa* | C_{3}H_{6}O | 152                    | Antimicrobial          |

Table 3. Antioxidant activities of the plant extracts.

| Antioxidant activity | *Moringa oleifera* | *Bidens pilosa* | Rutin | BHT | p-value |
|----------------------|--------------------|-----------------|-------|-----|---------|
| DPPH (%)             | 75.99 ± 1.12       | 77.14 ± 0.63    | 73.86 ± 0.84 | 70.67 ± 0.19 | 0.002   |
| ABTS (%)             | 82.8 ± 1.05        | 83.24 ± 0.67    | 79.3 ± 1.34  | 84.95 ± 0.43  | 0.12    |

Means within the same row having different superscripts were significantly different (p < 0.05).

DPPH: 2, 2-Diphenyl-2-picrylhydrazyl, ABTS: 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, BHT: butylated hydroxytoluene.

Table 4. Carotenoid and chlorophyll contents of *Moringa oleifera* and *Bidens pilosa* leaf extracts.

| Compounds | *Moringa oleifera* | *Bidens pilosa* | p-value |
|-----------|--------------------|-----------------|---------|
| Carotenoid (mg/g DW) | 0.39± 0.00 | 0.73± 0.00 | 0.001   |
| Chlorophyll a (mg/g DW) | 1.16± 0.01 | 2.62± 0.05 | 0.001   |
| Chlorophyll b (mg/g DW) | 0.31± 0.00 | 0.99± 0.01 | 0.001   |
| Total Chlorophyll (mg/g DW) | 1.46± 0.01 | 3.61± 0.04 | 0.001   |

Means within the same row having different superscripts were significantly different (p < 0.05).

Table 5. Effect of *Moringa oleifera* (ML) and *Bidens pilosa* (BP) leaf extracts on pH and TBARS values during refrigeration storage (4°C).

| Parameters | Treatment | Storage days | Control | 0.5 ML | 0.5BP | 1 ML | 1BP | 1MLBP | BHT | p-value |
|------------|-----------|--------------|---------|--------|-------|------|-----|-------|-----|---------|
| pH         |           | 0            | 5.18 ± 0.06 | 5.28 ± 0.12 | 5.28 ± 0.16 | 5.31 ± 0.10 | 5.30 ± 0.15 | 5.27 ± 0.18 | 5.37 ± 0.11 | 0.19    |
| TBARS (μgMDA/g) |           | 0            | 1.14 ± 0.21 | 1.10 ± 0.16 | 0.94 ± 0.20 | 0.92 ± 0.18 | 0.85 ± 0.17 | 1.09 ± 0.13 | 0.98 ± 0.10 | 0.28    |

Means within the same row having different superscripts were significantly different (p < 0.05).

*p* = 0.5 g/kg extracts, 1 = 1 g/kg extracts, Control = no antioxidant, 0.5 ML and 1 ML = *Moringa oleifera* leaf extracts; 0.5BP and 1BP = *Bidens pilosa* leaf extracts, 1MLBP = *Moringa oleifera* + *Bidens pilosa* leaf extract, BHT = butylated hydroxytoluene.

*p* = extracts of 0.5 g/kg, 1 = extracts of 1 g/kg, Control = no antioxidant, 0.5 ML y 1 ML = extractos de hoja de *Moringa oleifera*; 0.5BP y 1BP = extractos de hoja de *Bidens pilosa*; 1MLBP extractedos de hoja de *Moringa oleifera* + *Bidens pilosa*, BHT = butilhidroxitoluenu.
the treatment, indicating that the pH of the extracts did not affect the pH of the meat sample. However, all the beef samples treated with plant extracts had lower pH values compared with control and BHT treatments during the storage period. Similar results have been reported by Aytul, Korel, Arserim-Uçar, Uysal, and Bayraktar (2008) in raw beef meat treated with olive leaf extract and Muthukumar et al. (2014) in raw pork patties treated with Moringa oleifera extracts. This result, however, is in contrast to the findings of Shah et al. (2015) who observed significant change in the pH of modified atmosphere packaged raw beef treated with Moringa oleifera leaf extracts.

Effect of moringa oleifera and biden pilosa leaf extracts on TBARS

Oxidation is the main non-microbial cause of quality deterioration in processed meat products during storage (Falowo et al., 2014). The results of TBARS analysis showed that application of M. oleifera and B. pilosa extracts can protect ground beef against lipid oxidation during the storage period (Table 5). Although, the effect of the leaf extracts on TBARS values in ground beef were not statistically different (p > 0.05) compared with control during the storage periods. However, at day 6, the overall TBARS values of the beef samples containing extracts were lowered (ranging from 0.85 ugMDA/g to 0.94 ugMDA/g) compared with the control and BHT treatments at 1.14 ugMDA/g and 0.98 ugMDA/g, respectively. The inhibitory effects of these extracts against TBARS formation could be attributed to the inherent phyto-constituents and antioxidant activity as mentioned above. Other study has reported a positive correlation between phytochemical content or antioxidant activity of plant extracts and reduction in lipid oxidation in meat products (Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007). However, the addition of B. pilosa extracts exhibited higher antioxidant activity at 0.5 and 1 g/kg than the M. oleifera extracts. Possible reasons for higher antioxidant activity of B. pilosa extract on TBARS values could be linked to higher inherent phytochemicals and the presence of antioxidant compound such as Phenol, 2, 2'-methylenebis [6- (1, 1-dimethylethyl)]-4-methyl (BHT) which is absent in M. oleifera extracts. Our results is in line with the findings of Muthukumar et al. (2014) and Shah et al. (2015) who reported a lower TBARS values in raw pork and beef patties treated with Moringa plant extract.

Conclusion

This study has revealed that both M. oleifera and B. pilosa leaf extracts have substantial amounts of phytochemicals with significant free radical scavenging activity. The application of the M. oleifera and B. pilosa leaf extracts at 0.5 and 1 g/kg concentration can delay the formation of lipid oxidation in meat products during refrigerated storage. It also showed that the antioxidant potential of B. pilosa is much greater than M. oleifera. Moreso, both M. oleifera and B. pilosa leaf extracts could be used as a potential source of antioxidants to replace synthetic antioxidant in meat industry

Disclosure statement

No potential conflict of interest was reported by the authors.

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