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Original article

Moringa oleiferia leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties

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Summary The effective utilisation of *Moringa oleiferia* mature leaves (MOL) extract as an antioxidant in cooked goat meat patties during refrigerated storage was investigated, and its efficiency was evaluated against butylated hydroxytoluene (BHT). The extract exhibited high phenolic content (48.36 mg of gallic acid equivalent per g), flavonoid (31.42 mg g^{-1} of sample) being the major component. *Moringa oleiferia* mature leaves extract showed excellent antioxidant activity as determined by radical-scavenging activity of 1, 1-diphenyl 2 picrylhydrazyl (DPPH). The IC_{50} value of MOL extract for 2, 2-diphenyl-1-picrylhydrazyl radical scavenging was 18.54 μg mL^{-1}. Total phenolic content (as gallic acid equivalent) significantly ($P < 0.05$) increased from 285.56 in control to 379.45 in patties with MOL extract. MOL extract (0.1%) when added to meat was found to retard lipid peroxidation of cooked goat meat patties as measured by TBARS number during refrigerated storage. The increase in TBARS number in MOL extract–treated samples was very slow and remained lowest (0.53 mg malonaldehyde per kg sample) up to 15 days. The antioxidant activity of MOL extract was found to be comparable to BHT. Addition of MOL extract did not affect any of the sensory attributes of patties. The MOL extract at a level of 100 mg/100 g meat was sufficient to protect goat meat patties against oxidative rancidity for periods longer than the most commonly used synthetic antioxidant like BHT.

Keywords Extract, goat meat patties, lipid oxidation, *Moringa oleiferia*, natural antioxidant.

Introduction

Development of various comminuted meat products offers a profitable utilisation of tough meat. However, minced meat tends to become more rancid and brown rapidly, because of pigment and lipid oxidation. This problem is a major cause of muscle food deterioration as it decreases nutritional properties of foods. Lipid peroxidation results in the formation of reactive oxygen species and free radicals, which are purportedly associated with carcinogenesis, mutagenesis, inflammation, DNA changes, ageing and cardiovascular diseases. In addition, lipid oxidation affects essential sensory traits of meat product, causing flavour, colour and texture deterioration (Estevez *et al*., 2005). Moreover, mechanisms for control of lipid oxidation have become increasingly important with the rise in popularity of precooked and convenience foods (Das *et al*., 2006). The use of antioxidants in lipids and lipid-containing foods is one method to minimise rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) are widely used in the food industry because they are more effective and less expensive than natural antioxidants. Their safety, however, has been questioned. TBHQ is banned in Japan and certain European countries, and BHA and BHT are reported to be carcinogenic. Hence, research into safer and more effective natural antioxidants is under way, and several natural sources are being examined. Natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic value. The increased interest in natural antioxidants has led to the antioxidant evaluation of many species of fruits, vegetables, herbs, spices and cereals (Wolfe *et al*., 2003; Liyana-Pathirana & Shahidi, 2005). For this reason, there is growing interest in separating these plant antioxidants and using them as natural antioxidants. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive.

Moringa oleifera is the most widely cultivated species of a monogeneric family; the Moringaceae that is native

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to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan and is commonly known as horse radish tree or drumstick tree. It has both nutritional and medicinal values with some useful minerals, vitamins, amino acids, etc. (Ramachandran *et al*., 1980; Sánchez-Machado *et al*., 2010). Almost all parts of this plant like root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatorenal disorders (Morimitsu *et al*., 2000). In many reports, the *M. oleiferia* is described as an ornamental plant with medicinal, therapeutic or healing properties. The flowers and roots are used in folk remedies, the seeds are used for abdominal tumours; leaves are applied as poultice to sores, rubbed on temples for headaches and are said to have purgative properties (Anwar *et al*., 2007). The young leaves are used as fresh green vegetable and are commonly cooked and eaten like spinach as well as to make soup or salads in many parts of India. The leaves are an exceptionally good source of protein, provitamin A, vitamins B and C, minerals (particularly iron) and rich source of essential amino acids such as methionine, cystine, tryptophan and lysine (Makkar & Becker, 1997; Sánchez-Machado *et al*., 2010). Moringa preparations have been cited in the scientific literature as having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolaemic and hypoglycaemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of Schistosome cercariae titre (Fahey, 2005). The Moringa plant provides various bioactive compounds like glucosinolates and isothiocyanates and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol, and the stem bark has been reported to contain alkaloids namely moringinine and moringine (Fahey *et al*., 2001). It has been reported that Moringa leaves significantly increase the shelf life of ghee, and such improvement of shelf life of ghee may be due to various types of natural antioxidant compounds such as ascorbic acid, carotenoids and phenolic substances, which are present in Moringa leaves (Siddhuraju & Becker, 2003). Recently, the immunomodulatory activity of *M. oleiferia* extract has been also reported, and the results indicate that leaf extract significantly reduced cyclophosphamid-induced immunosuppression in mice by stimulating both cellular and humoural immunity (Gupta *et al*., 2010). Although much has been learned about the nutritional value of *M. oleiferia,* additional knowledge remains to be secured. Additionally, its use as an antioxidant in cooked meat to control lipid peroxidation has not been studied. Therefore, this work was undertaken to examine the utilisation of *M. oleiferia* leaves extract, as a source of natural antioxidants and its effectiveness in reducing lipid peroxidation of cooked goat meat patties.

Materials and methods

Ingredients

Goat meat was procured from the experimental slaughter house of Goat Products Technology Lab, CIRG, Makhdoom. It was kept under frozen storage at −18 °C till the product processing. Chemicals used in the experiment are BHT, DPPH, gallic acid and caffeic acid (Sigma Chemical Co., Bangalore, India), thiobarbituric acid (BDH Chemicals Ltd., Mumbai, India), and HPLC-grade methanol (Merck, India). All other reagents used were of analytical grade and procured from S. D. Fine Chemicals (Mumbai, India) and Qualigens Fine Chemicals (Mumbai, India).

Preparation Moringa leaves extract

To prepare leave extract, fresh mature leaves of *M. oleiferia* were collected from the Horticulture Section of the Institute. The leaves were properly cleaned, chopped to small pieces and dried in shade. The dried leaves were powdered, passed through sieve no. 20 and extracted (100 g) successively with 600 mL of water in a Soxhlet extractor for 18–20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40–50 °C). The yield (w/w) of the extract from fresh leaves was about 8–9%. The extract was prepared in duplicate, and all analysis was carried out in triplicates.

Detailed study

In the present experiment, Moringa leave extract was used (0.1%) as a source of natural antioxidant in the goat meat patties (MOL Patties). Its antioxidant activity was evaluated against 0.1% BHT (BHT Patties) added and goat meat patties without antioxidants (Control Patties). The formulation of the three products is given in the Table 1.

Preparation of emulsion and goat meat patties

Goat meat was double minced (10-mm plate followed by 8-mm plate) using a meat mincer (Tallers Ramon Model P-22, Barcelona). Minced meat and all the ingredients were thoroughly mixed and chopped by a bowl chopper (Seydelmann K20 Ras, Germany) to prepare the emulsion for each treatment. About 70 g of emulsion was moulded in a Petri dish (75 mm diameter and 15 mm height) to form patties and was cooked in a preheated oven (Narang Scientific Works, New Delhi, India) at 180 ± 5 °C for 15 min, after which they were turned

Table 1 Formulation for goat meat patties with *Moringa oleiferia* leaf extract and butylated hydroxyl toluene (BHT)

Ingredients	Control patties	MOL patties	BHT patties
Meat (%)	69.8	69.7	69.7
Sodium chloride (%)	1.2	1.2	1.2
Sodium tripolyphosphate (%)	0.5	0.5	0.5
Sodium nitrite (ppm)	150	150	150
Ice flakes (%)	12	12	12
Refined vegetable oil (%)	7	7	7
MOL extract (%)	–	0.1	
BHT (%)	–	–	0.1
Condiment mix (%)	5	5	5
Dry spice mix (%)	1.5	1.5	1.5
Refined wheat flour (%)	3	3	3

MOL, *Moringa oleiferia* leaves.

and allowed to get cooked for 10 more minutes till internal temperature reached about 75 ± 2 °C recorded by a probe thermometer (Oakton, China). After cooling to room temperature, the patties were aerobically packaged in a low-density polyethylene pouches and stored at 4 °C for 15 days and analysed for total phenolic content, pH, sensory attributes and thiobarbituric acid reactive substances (TBARS) number. All the parameters and sensory evaluation were done only on day 0 except the TBARS values, which were analysed throughout the storage.

Analysis of *M. oleiferia* leaf extract and goat meat patties

Estimation of total phenolics

The concentration of phenolic compounds in the plant extracts was determined by the Folin–Ciocalteu method as described by Singleton & Rossi (1965). *Moringa oleiferia* leaf extract was dissolved in distilled water, and to 1 mL of sample, 5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water) was added. After 5 min, 4 mL of a sodium carbonate solution (7.5%) was added to each tube. The tubes were incubated for 2 h at room temperature, and the absorbance determined spectrophotometrically (U-28000 Spectrophotometer; Hitachi, Tokyo, Japan) against a reagent blank at 725 nm. A standard curve was plotted using different concentrations of gallic acid, and the amount of total phenolics was calculated as gallic acid equivalents in mg g^{-1} of plant materials. Total phenolics in cooked goat meat patties was analysed using the Folin–Ciocalteu (F-C) assay (Escarpa & Gonzalez, 2001) with slight modifications. Five gram of cooked patty was homogenised with 25 mL of 70% acetone and kept overnight for extraction at refrigeration temperature. Suitable aliquots of extracts were taken in a test tube, and the volume was made to 0.5 mL with distilled water followed by the addition of 0.25 mL F-C (1 N) reagent and 1.25 mL sodium carbonate solution (20%). The tubes were vortex mixed, and the absorbance was recorded at 725 nm after 40 min.

Estimation of total flavonoids

Total flavonoids were measured by a colorimetric assay developed by Zhishen *et al.* (1999). One millilitre aliquot of appropriately diluted sample or standard solutions of catechin (20, 40, 60, 80 and 100 mg L^{-1}) was added to a 10-mL volumetric flask containing 4 mL H_2O. At zero time, 0.3 mL 5% NaNO was added to the flask. After 5 min, 0.3 mL 10% AlCl3 was added. Finally, at 6 min, 2 mL 1 M NaOH was added to the mixture. Immediately, the mixture in the reaction flask was diluted to volume with the addition of 2.4 mL of H_2O and thoroughly mixed. Absorbance of the mixture (pink in colour) was determined at 510 nm versus blank prepared with water. Total flavonoids of leaf extract were expressed as mg flavonoids per g plant materials.

Measurement of reducing power

The reducing power of the extracts was determined according to the method of Yen & Duh (1993). Different concentrations of *M. oleiferia* leaf extract in 1 mL methanol were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide in 10-mL test tubes. The mixtures were incubated for 20 min at 50 °C followed by the addition of 2.5 mL of 10% trichloroacetic acid and then centrifuged at 9700 ***g*** for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of ferric chloride (0.1% w/v), and the absorbance was measured at 700 nm (U-28000 Spectrophotometer; Hitachi). Increase in absorbance of the reaction mixture indicated the reducing power of the sample.

Radical-scavenging activity using DPPH assay

The DPPH assay was performed according to the method of Yamaguchi *et al.* (1998). An aliquot of the extract (200 μL) was mixed with 800 μL of Tris-HCl buffer (100 mM, pH 7.4). To this, 1 mL of 500 μM DPPH in ethanol (final concentration of 250 μM) was added, and the mixture was vortexed vigorously. The tubes were then incubated at room temperature for 20 min in the dark, and the absorbance was taken at 517 nm. The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity}\% = (\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}}/\text{Absorbance}_{\text{Blank}}) \times 100$$

pH and cooking yield of patties

The pH of the cooked patty was determined by blending 10-g sample with 50 mL distilled water for a minute in a homogeniser (Model PT-MR-2100; Kinematica AG, Switzerland). The pH values were measured using a standardised electrode attached to a digital pH meter (μ pH system 361; Systronics, Delhi, India). Cooking yield was determined by dividing the weight of cooked product by the weight of raw uncooked meat batter and expressed as per cent.

Thiobarbituric acid reactive substances (TBARS) number

Lipid oxidisation in the goat meat patties was monitored by measuring thiobarbituric acid reactive substances at an interval of 5 days during refrigerated storage. The TBARS number (mg malonaldehyde PER kg) of the goat meat patties was determined using the extraction method described by Witte *et al.* (1970) with slight modifications, as the slurry was centrifuged at 3000 ***g*** for 10 min (Biofuge Primo R, Heraeus, Germany) instead of filtration through Whatman No. 42.

Sensory analysis of goat meat patties

The sensory attributes such as appearance, flavour, texture, juiciness and overall palatability of the product were evaluated using 8-point descriptive scale (Das *et al.*, 2008), where eight corresponded to 'components characteristic of the highest quality'. Scores from 8 to 5 were considered acceptable. The panel consisted of 10–12 trained and experienced members of the CIRG, staff who were familiar with the characteristics of meat product. Patties were warmed in a microwave oven for 20 s just before sensory evaluation, and coded samples were served at room temperature in separate booths. Water was served for cleansing the mouth between samples.

Statistical analysis

Three replications of the study were performed, and measurements of all parameters were taken in duplicate. Mean values for various parameters were calculated and compared by analysis of variance using the SPSS software for Windows (IBM Corporation, NY, USA; version 13.0). Means of pH, DPPH, total phenolics and flavonoids and sensory attributes were analysed using one-way ANOVA. Storage data of TBARS values were analysed using two-way ANOVA with treatment and storage time as main effects. Statistical significance was identified at the 95% confidence level ($P < 0.05$). The values were presented as mean along with standard error (mean ± SD).

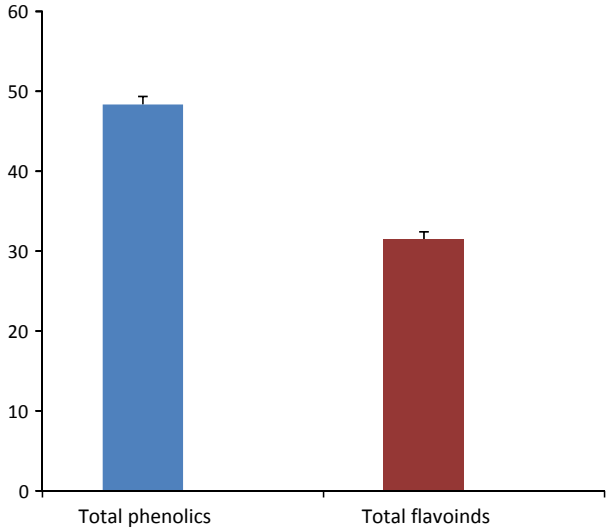

Figure 1 Total phenolics and flavonoid contents in *Moringa oleiferia* leaf extract[a]. [a]Each value is the mean ± SE of three replicates experiments. Phenolics expressed as mg gallic acid equivalents (GAE/g of plant material). Flavonoids expressed as mg equivalent of catechin per g plant material.

Results and discussion

Total phenolics and flavonoid content

As the extract used in goat meat products, water was used as extracting solvent. Although water may not be the efficient reagent in extracting the antioxidants from MOL, methanol, ethanol and butanol could be used. But care should be taken that no residual solvent is left as this could be toxic when added to food. Total phenolics and flavonoids content of aqueous extract of *M. oleiferia* leaves are presented in Fig. 1. Sreelatha & Padma (2009) have reported the total phenolic acid content in MOL extract to be 45.81 mg g^{-1}, and Kaur & Kapoor (2002) reported the total phenolic content of *Trigonella foenum graecum* to be 217.5 mg of catechol/100 g of fresh vegetable. Total polyphenol content of some common Indian green leafy vegetables was found to be in the range of 5–65.5 mg tannic acid per g of extract (Shyamala *et al.*, 2005). Major difference in total phenolic content depends on the stage of maturity and solvent used for extraction. The flavonoids content of MOL extract was 22.4 mg g^{-1} in terms of catechin equivalent. Mature MOL extract was also reported to be a rich source of flavonoids (Sreelatha & Padma, 2009).

DPPH radical-scavenging activity

DPPH (1, 1-diphenyl-2picrylhydrazyl) assay has been widely used to evaluate the free radical–scavenging ability of various plant extracts. DPPH having maxi-

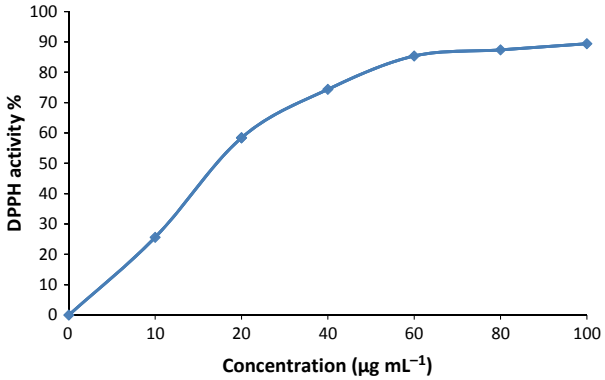

Figure 2 DPPH activity of the *Moringa oleiferia* leaves extract.

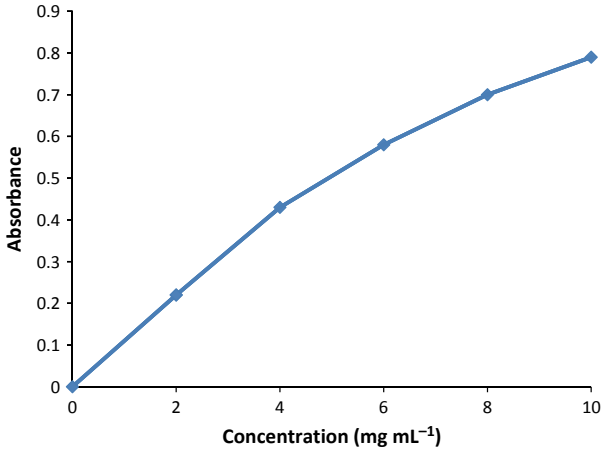

Figure 3 Reducing power of the *Moringa oleiferia* leaves extract.

mum absorption at 517 nm is a stable free radical. In the presence of an antioxidant, it accepts an electron or hydrogen atom, and thus, there is a decrease in absorbance. The result of radical-scavenging activity of MOL extract at different concentration is shown in Fig. 2. Increasing the concentration of the extract significantly increase the radical-scavenging activity. MOL extract showed a concentration-dependent DPPH radical-scavenging activity with an IC50 of 18.54 μg mL^{-1}. Similar results were reported by Sreelatha & Padma (2009) who found that MOL extract significantly reduced DPPH radicals with an increase in the concentration.

Reducing power

Different studies have indicated that the electron donation capacity (reflecting the reducing power) of bioactive compounds is associated with antioxidant activity (Yen *et al*., 1996; Siddhuraju *et al*., 2002). In this assay, the ability of extracts to reduce iron (III) to iron (II) was determined and compared with that of ascorbic acid, which is known to be a strong reducing agent. The reducing power of a compound serves as a significant indicator of its potential antioxidant activity. With increasing concentration, there was an increase in absorbance of MOL extract (Fig. 3). MOL extract showed a high reducing power of 0.79 optical density, even at a concentration of 10 mg mL^{-1}. The reducing power of the aqueous extract of MOL increased with increasing concentrations of the extract. Among the green leafy vegetables, *M. koenigii* had the highest reducing power as estimated using the potassium hexacyanoferrate method at concentrations of 2–10 mg mL^{-1}. The reducing power of green leafy vegetables followed the order *M. koenigii* > *T. graecum* > *Amaranthus* sp. > *C. asiatica* (Gupta & Prakash, 2009).

pH, cooking yield and phenolic content of patties

The pH of raw and cooked goat meat patties, cooking yield and total phenolic content of cooked patty is given in Table 2. Use of extract and BHT did not influence the pH of raw and cooked goat meat patties, but raw patties with BHT had lower pH value compared with the others. Naveena *et al*. (2008) reported that chicken patties with BHT had lower pH value compared with chicken patties with pomegranate juice and rind powder. There was no significant difference in cooking yield between control and treated patties. The total phenolic content of cooked goat meat patties with MOL extract

Table 2 Effect of MOL extract and BHT addition on the pH, cooking yield and total phenolics content

Treatment groups	Raw pH	Cooked pH	Cooking yield (%)	Total phenolics (as gallic acid eq) μg g^{-1}
Control patties	6.12 ± 0.04	6.24 ± 0.11	89.23 ± 1.23	285.56 ± 8.56[b]
MOL patties	6.10 ± 0.02	6.22 ± 0.14	89.79 ± 0.86	379.45 ± 10.25[a]
BHT patties	6.08 ± 0.01	6.20 ± 0.13	88.92 ± 1.09	296.24 ± 7.38[b]

MOL, *Moringa oleiferia* leaves; BHT, butylated hydroxyl toluene.
Number of observations = 6.
Mean value in the same column bearing the same superscript do not differ significantly ($P < 0.05$).

was significantly ($P < 0.05$) higher compared with control and BHT patties. The higher level of phenolics may be the indication that this product is nutritionally enriched/fortified with the addition of MOL extract. Leheska *et al.* (2006) observed an increase in phenolic content of precooked pork breakfast sausages prepared with fruit purees, and Naveena *et al.* (2008) have also reported that pomegranate rind powder (PRP) significantly increased the total phenolic content of cooked chicken patties. The phenolic compounds are of great interest as they are involved in biochemical and pharmacological effects, including anticarcinogenic and antioxidant effects (Doshi *et al.*, 2006).

Lipid peroxidation in goat meat patties

As MOL demonstrated good antioxidant activity, its efficacy in retarding lipid peroxidation of cooked goat meat patties was examined. Effect of MOL extract treatment on thiobarbituric acid reactive substances (TBARS) number in cooked goat meat patties is shown in Fig. 4. The MOL and BHT treatments significantly ($P < 0.05$) reduced the TBARS number compared with control throughout the storage. The increase in TBARS number in MOL extract–treated samples was very slow and remained lowest (0.53 mg malonaldehyde per kg sample) up to 15 days. Initial TBARS number of cooked goat meat patties containing MOL extract was 30% less than the corresponding sample not containing extract. Over the storage period, a 47% reduction was obtained in cooked goat meat with MOL extract. Thus, the efficacy of MOL extract in retarding oxidative rancidity in cooked goat meat samples was evident and comparable to that of BHT. Sebranek *et al.* (2005) observed that the rosemary extract was more effective than BHA/BHT for preventing increased TBARS values of precooked frozen pork sausage. The large amount of phenolics contained in mature MOL extract may be the reason for its strong antioxidant ability (Sreelatha & Padma, 2009). Antioxidant activity of phenolic compounds is mainly because of their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet oxygen or decomposing peroxides (Cao *et al.*, 1997). The mode of action of MOL extract may be in similar fashion, i.e. by donating electrons and reacting with free radicals to convert them to more stable products and terminate free radical chain reactions. The data indicated that the marked antioxidant activity of MOL extract seems to be the result of their radical-scavenging activity and reducing power. Natural antioxidants are believed to break free radical chains of oxidation by donation of an hydrogen from the phenolic groups, thereby forming a stable end product (Sherwin, 1998).

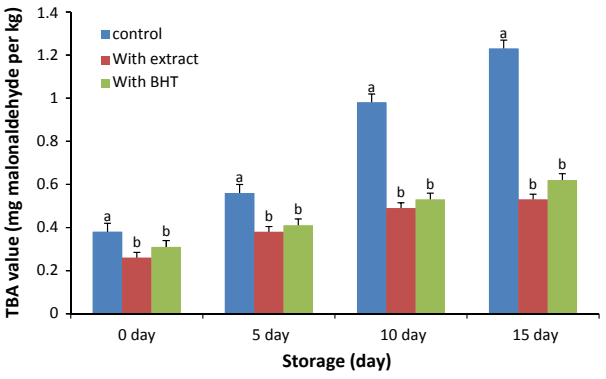

Figure 4 Effect of *Moringa oleiferia* mature leaves extract incorporation on the TBARS number of goat meat patties stored at 2–4 °C. Means with the same letter (a, b or c) are not significantly different ($P > 0.05$).

Sensory evaluation of patties

Sensory analysis of the fresh goat meat products revealed that addition of MOL extract had no effect on the sensory attributes. With respect to colour, flavour, taste and texture, the MOL extract–treated and control samples were similar. After 15 days of chilled storage, goat meat patties containing 0.1% MOL extract was sensorially acceptable, and addition of extracts did not have any negative effect on sensory attribute. Devatkal *et al.* (2010) observed that addition of extracts of kinnow rind powder, pomegranate rind powder and pomegranate seed powder did not have any negative effect on sensory attributes of cooked goat meat patties. Therefore, MOL extract at 0.1% has the potential to reduce oxidative rancidity and improve shelf life of refrigerated cooked goat meat patties.

Conclusion

Mature *M. oleiferia* leaves are good sources of phenolic compounds and have very potent antioxidant activity. Incorporation of 0.1% extract of MOL (100 mg/100 g meat) could protect cooked goat meat patties against lipid oxidation during refrigerated storage. The MOL extract was more effective than BHT in maintaining low TBARS number of precooked chilled goat meat patties. In Indian cities, frozen ready-to-eat meat products are available, but freezing is quite expensive leading to limited market. Therefore, MOL-treated meat products that can be stored at chilled temperatures would be beneficial for the manufacturer as well as the consumer. Unutilised mature Moringa leaves are the potent source of phenolics and have immense nutraceutical value for the development of functional meat products of commercial interest.

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