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Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat

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Abstract

The effectiveness of mint leaves, a common herb used in Indian cuisine, as a natural antioxidant for radiation-processed lamb meat was investigated. Mint extract (ME) had good total phenolic and flavonoid contents. It exhibited excellent antioxidant activity, as measured by b-carotene bleaching and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. It also showed a high superoxide- and hydroxyl-scavenging activity but low iron-chelating ability. A positive correlation was found between the reducing power and the antioxidant activity. The antioxidant activity of ME was found to be comparable to the synthetic antioxidant, butylated hydroxytoluene (BHT). The suitability of ME as an antioxidant was determined during radiation processing of lamb meat. ME retarded lipid oxidation, monitored as thiobarbituric acid-reactive substances (TBARS), in radiation-processed lamb meat. TBARS values of ME containing irradiated meat stored at chilled temperatures were significantly lower ($p < 0.05$) than samples without ME. After 4 weeks of chilled storage, TBARS in irradiated meat containing ME (0.1%) was half of that in untreated irradiated meat. $© 2005 Elsevier Ltd. All rights reserved.$

Keywords: Irradiation; Lipid oxidation; Antioxidants; Mint extract

1. Introduction

Microbial contamination of meat is a serious concern, for both meat producers and consumers. Use of radiation processing is gaining momentum as an alternate technology to eliminate microbial contamination. Several countries have approved irradiation of meat and meat products [\(Molins, Motarjemi, & Kaferstein, 2001](#page-7-0)). However, wide acceptability of radiation-processed meat products will depend upon quality parameters, such as oxidative changes, colour stability and organoleptic attributes. Irradiation is known to accelerate lipid peroxidation of meat [\(Formanek, Lynch, Galvin, Farkas, & Kerry,](#page-6-0) [2003](#page-6-0)). Lipid oxidation is responsible for the loss of quality in meat besides microbiological deterioration. It occurs during processing and storage of meat and meat products. Products of lipid peroxidation adversely affect the colour, flavour, texture and nutritive value of meat. It is therefore necessary to control these changes for better product development.

Addition of antioxidants is one of the simplest means of ensuring oxidative stability of irradiated meat. The growing interest in substitution of synthetic antioxidants by natural antioxidants has fostered research on screening of plant materials in order to identify new compounds. Antioxidants have also received increased attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases ([Liu, 2003\)](#page-7-0).

Attempts are being made in our laboratory to explore the use of novel natural antioxidants for radiation-processed meat and meat products [\(Kanatt, Chander, &](#page-7-0) [Sharma, 2004, 2005](#page-7-0)). Mentha spicata L., commonly known as spearmint or garden mint, is often used in Indian cuisine. It is popularly consumed in the form of mint chutney and added to several meat preparations as a flavour

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enhancer. The genus Mentha belongs to the family Lamiaceae (Labiatae), consisting of about 25–30 species ([Ali,](#page-6-0) [Saleem, Ahmad, Parvez, & Yamdagni, 2002\)](#page-6-0). This family is a rich source of polyphenolic compounds and hence could possess strong antioxidant properties. Members of the genus are characterized by their volatile oils that are of great economic importance, being used by the pharmaceutical, cosmetic, food, confectionery and liquor industries. Hence, they are cultivated as industrial crops in several countries. There are a few reports on the antioxidant property of Mentha ([Dorman, Kosar, Kahlos, Holm,](#page-6-0) [& Hiltunen, 2003; Marinova & Yanishlieva, 1997; Zheng &](#page-6-0) [Wang, 2001\)](#page-6-0) but its application in meat has not been studied. The aims of the present study were to investigate the antioxidant potential of Mentha spicata L., and to test its efficacy in minimizing oxidative rancidity of radiation-processed lamb meat.

2. Materials and methods

2.1. Chemicals

b-Carotene, linoleic acid, nitroblue tetrazolium (NBT), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and catechin were purchased from Sigma Chemical Co. (St. Louis, MO). Thiobarbituric acid (TBA) was obtained from BDH Chemicals Ltd. (Poole, England). Phenazine methosulphate (PMS), deoxyribose, nicotinamide adenine dinucleotide (NADH) disodium salt and ferrozine were purchased from HiMedia (Mumbai, India). All other reagents used were of analytical grade and procured from Qualigens Fine Chemicals (Mumbai, India) or Sisco Research Lab (Mumbai, India).

2.2. Preparation of mint extract (ME)

Mint (Mentha spicata L.) was purchased from a local market. The leaves were separated and washed under tap water. Mint leaves (100 g) were refluxed with distilled water (1000 ml) for 1 h. Using cheesecloth, the filtrate was separated and further filtered using Whatman No. 4 filter paper. The residue was then extracted with an additional 1000 ml of distilled water, as described above. The extracts were then pooled and centrifuged at 12,100g for 20 min. The supernatant was again filtered (Whatman 4) and concentrated using a rotary evaporator (Buchi Rotavapor, Flawil, Switzerland). The concentrate was then lyophilized to form a powder and stored at 4° C until analyzed.

2.3. Determination of total phenolics and flavonoids

The amount of total phenolics in the extracts was determined according to the Folin-Ciocalteu procedure ([Single](#page-7-0)[ton & Rossi, 1965\)](#page-7-0). Appropriately diluted ME was mixed with Folin-Ciocalteu reagent $(0.2 N)$ and allowed to stand at room temperature for 5 min. Sodium bicarbonate solution (6%, 0.75 ml) was added to the mixture and incubated at room temperature for 90 min. Absorption at 725 nm was measured using a spectrophotometer (Varian DMS 100, Australia). The total phenolic content was expressed as catechin equivalents. The determination of flavonoids was performed according to the colorimetric assay of [Kim,](#page-7-0) [Jeong, and Lee \(2003\).](#page-7-0) To 1 ml of diluted ME, 4 ml of distilled water were added. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminium chloride solution (0.3 ml). Tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as catechin equivalents.

2.4. Determination of antioxidant activity

The antioxidant capacity was estimated by thermally induced β -carotene bleaching assay, as described by [Velio](#page-7-0)[glu, Mazza, Gao, and Oomah \(1998\)](#page-7-0) with some modifications. The assay reagent was prepared by mixing of $chloroform$ solution of β -carotene, linoleic acid and Tween 80. Chloroform was then removed under a stream of nitrogen and, to this mixture, distilled water (100 ml) was added. The aliquots of this reagent were transferred into a series of tubes containing the ME. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. The tubes were then incubated in a water bath at 50° C to induce autoxidation. The absorbance at 470 nm was recorded after 60 min. Antioxidant activity coefficient (AAC) was calculated as:

$$
AAC = \frac{A_{S(60)} - A_{C(60)}}{A_{C(0)} - A_{C(60)}} \times 100,
$$

where, $A_{S(60)}$ was the absorbance of the antioxidant mixture at $t = 60$ min, $A_{C(60)}$ the absorbance of the control at $t = 60$ min, and $A_{C(0)}$ the absorbance of the control at $t = 0$ min.

2.5. Radical scavenging activity

2.5.1. DPPH assay

DPPH assay was performed according to the method of [Yamaguchi, Takamura, Matoba, and Terao \(1998\)](#page-7-0). The diluted extract $(200 \mu l)$ was mixed with 800 μl of Tris–HCl buffer (100 mM, pH 7.4). To this was added 1 ml of 500 μ M DPPH in ethanol (final concentration of $250 \mu M$) and the whole vortexed vigorously. The tubes were then incubated at room temperature for 20 min under dark conditions and the absorbance was measured at 517 nm. Percent DPPH-scavenging activity was calculated as:

 $[(Control absorbance - Extract absorbance)/$

(Control absorbance) \times 100.

2.5.2. Site specific and non-site specific hydroxyl ionscavenging activity

Non-site specific hydroxyl radical scavenging activity of ME was determined according to the deoxyribose method of [Halliwell, Gutteridge, and Aruoma \(1987\)](#page-6-0) in the presence of EDTA. To 1 ml of appropriately diluted sample, 1 ml phosphate buffer (0.1 M pH 7.4) containing 1 mM ferric chloride, 1 mM EDTA, 1 mM ascorbic acid, 30 mM deoxyribose and 20 mM hydrogen peroxide, were added. Following incubation at 37 °C for 90 min, 2 ml of 2% (w/ v) TCA and 2 ml of 1% (w/v) TBA were added to the reaction mixture, which was then heated in a boiling water bath for 15 min. The absorbance of pink colour developed was measured at 532 nm using a spectrophotometer. Site-specific hydroxyl radical-scavenging activity of ME was performed, as described above, except that EDTA was absent in the reaction system. The percent inhibition of hydroxyl radical was calculated, as above, for DPPH assay.

2.5.3. Superoxide anion radical-scavenging activity

Superoxide anion-scavenging activity of ME was determined according to the method of [Liu, Ooi, and Chang](#page-7-0) [\(1997\)](#page-7-0) with some modifications. The reaction mixture consisted of 1 ml of NBT (156 μ M in 0.1 M potassium phosphate buffer pH 7.4), 1.0 ml of NADH (468 μ M in 0.1 M potassium phosphate buffer pH 7.4) and 0.5 ml of an appropriately diluted sample. The reaction was initiated by addition of 100 μ l of PMS (60 μ M in 0.1 M potassium phosphate buffer pH 7.4) to the mixture. The tubes were incubated at ambient temperature for 5 min and the absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% Inhibition = $[(A_0 - A_s)/A_0] \times 100$,

where A_0 is absorbance of the control and A_S is absorbance of the sample.

2.6. Iron chelation

The chelation of iron (II) ions by the ME was studied, as described by [Decker and Welch \(1990\)](#page-6-0). An aliquot of the extract (1 ml) was added to 100 μ l of 1 mM FeCl₂ and 3.7 ml of distilled water. The reaction was initiated by adding 200 µl of 5 mM ferrozine. After a 20 min incubation at room temperature, the absorbance at 562 nm was recorded. EDTA was used as a positive control. The control contained all the reaction reagents except the extract or positive control. The Fe^{2+} -chelating activity was calculated using the equation below:

Chelation activity(%) = [$(A_0 - A_s)/A_0$] × 100,

where, A_0 is the absorbance of control and A_S the absorbance of extract.

2.7. Reducing power

The reducing power of the extracts was determined according to the method of [Oyaizu \(1986\)](#page-7-0). An aliquot of ME (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50° C for 20 min. Ten percent TCA (2.5 ml) was added and the mixture was centrifuged at 650g for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm.

2.8. Effect of irradiation and heating on ME

ME (1%) was irradiated at various doses (2.5, 5, and 10 kGy) in a Gamma Cell 5000 at a dose rate of 4.5 kGy h^{-1} . ME (1% solution) was also autoclaved. Total phenolic content and DPPH-scavenging assays of these samples were then carried out as described above.

2.9. Antioxidant potential of ME in irradiated lamb meat

2.9.1. Meat sample preparation

The visible fat was removed from the leg region of lamb meat. It was then minced and divided into five lots. To one lot, ME (0.05%) was added, mixed thoroughly and then packed in polythene pouches and irradiated at 2.5 kGy. ME (0.1%) and BHT (0.02%) were added to the second and third lots, respectively, while no antioxidant was added to the fourth lot. All four lots were then processed like the first lot. Non-irradiated sample was kept as control. The samples were subjected to γ irradiation at melting ice temperature $(1-3 \degree C)$ in a Package Irradiator (Nordion Intl. Inc., Ontario, Canada) with a ${}^{60}Co$ source at a dose rate of 3.9 kGy h^{-1} . The samples received minimal doses of 2.5 kGy with an overdose ratio of 1.25. Dosimetry was performed by a cerric-cerrous dosimeter calibrated against Fricke's dosimeter. Dosimetry intercomparison was carried out with National Standards established by the Radiological Physics and Advisory Division (RP&AD), Bhabha Atomic Research Centre (BARC), Mumbai, India.

2.9.2. Measurement of lipid peroxidation

Thiobarbituric acid-reactive substances (TBARS) produced from lipid peroxidation were determined using the method of [Alasnier, Meynier, Viau, and Gandmer \(2000\).](#page-6-0) Meat sample was blended with 5% trichloroacetic acid (TCA) and BHT (10 μ g BHT/g of lipids). It was then filtered through a Whatman filter paper (No. 4). Equal amounts of filtrate and 0.02 M TBA were heated in a boiling water bath for 30 min, cooled and absorbance was measured at 532 nm. TBARS were expressed as mg of malonaldehyde per kg of meat.

2.10. Statistical analysis

Samples were assayed in triplicate and the results are shown as means \pm standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with Tukey's post test using GraphPad InStat version 3.05 for Windows 95, GraphPad Software, San Diego California USA, [www.graphpad.com.](http://www.graphpad.com) Differences at $p \leq 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Total phenolic and flavonoid contents

The extract yield was found to be 61.5 mg/g of leaves. Phenolics and polyphenolic compounds constitute the main class of natural antioxidants present in plants and are usually quantified by employing Folin's reagent. The total phenolic content of mint extract was found to be 25.62 ± 3.14 mg in terms of catechin equivalent/g of wet weight of the sample. [Dorman et al. \(2003\)](#page-6-0) have reported a total phenolic content in the range of 128–230 mg gallic acid equivalents/g (dry weight) of extract from different Mentha plants. [Triantaphyllou, Blekas, and Boskou](#page-7-0) [\(2001\)](#page-7-0) reported that the extracts of Mentha species contained bound phenolic acids and flavonoids. The major phenolic acids reported in water-soluble Mentha spicata extract are eriocitrin, luteolin glucoside, rosmarinic acid and caffeic acid ([Dorman et al., 2003\)](#page-6-0).

The most widespread and diverse phenolics are the flavonoids which are polyphenolic secondary metabolites widely dispersed throughout the plant kingdom. The total flavonoid content of ME was 13.5 ± 1.38 mg in terms of catechin equivalents/g of the sample. Flavone aglycones such as thymonin, diosmetin, have been reported in Mentha spicata ([Subramanian & Nair, 1972\)](#page-7-0). There is a wide degree of variation between different phenolic compounds in their effectiveness as antioxidants ([Robards, Prenzler,](#page-7-0) [Tucker, Swatsitang, & Glover, 1999](#page-7-0)). The number of hydroxyl groups and substitution with electron-donating alkyl or methoxy groups of flavonoid increases the antioxidant potential.

3.2. Antioxidant activity of ME

In view of the diversity among the number of antioxidant assays available, the results of a single assay can only suggest the antioxidant property of the plant extract. Hence, multiple assays are necessary to ensure better comparison of the results, covering a wider range of possible applications and thus to unequivocally prove the antioxidant property of an extract. From the plethora of antioxidant tests available, those methods that allow both the primary and secondary steps of oxidation and the lipid-soluble antioxidant capacity to be evaluated will be more desirable. Plant-derived phenolic compounds are known to exhibit antioxidant activity through a variety of mechanisms, including scavenging of reactive oxygen species, inhibiting lipid peroxidation and chelating of metal ions [\(Shahidi, 1997](#page-7-0)). Hence, a number of in vitro antioxidant assays were employed to determine the antioxidant capacity of ME and to gain insight of its possible mechanism.

$3.2.1.$ β -Carotene bleaching assay

 β -Carotene bleaching assay has a high specificity for lypophilic compounds. Fig. 1 shows the antioxidant activity coefficient of BHT and ME. Concentration significantly $(r^2 = 0.94, p < 0.05)$ influenced the antioxidant potential of the samples. At a concentration of $250 \mu g$ ME had an AAC of 329, while the corresponding AAC for BHT was 740. At 1 mg concentration an AAC of 682 was obtained for ME, whereas BHT displayed an AAC of 900. BHT, being a synthetic antioxidant, however, cannot be used beyond a concentration of 200 ppm [\(Suja, Jayalekshmy,](#page-7-0) [& Arumughan, 2005](#page-7-0)) while, for antioxidants from natural sources, there is no such limit.

3.2.2. DPPH radical-scavenging activity

Several free radicals, such as OH^{\bullet} , $O_2^{\bullet-}$, LOO' having different reactivities are formed during lipid oxidation. Relatively stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity [\(Jao & Ko, 2002](#page-7-0)). [Fig. 2](#page-4-0) shows the percentage scavenging activity of mint extract of the DPPH free radical. Mint extract showed a strong correlation between its antioxidant activity and concentration $(r^2 = 0.99)$, $p < 0.05$). The IC₅₀ value of ME, which is the concentration at which 50% scavenging of the free radical is obtained, was found to be $25.8 \mu g/ml$, whereas, that of the synthetic antioxidant BHT was $10.1 \mu g/ml$. In case of coriander leaves, another commonly used herb in Indian cuisine, a

Fig. 1. Antioxidant activity of ME by b-carotene bleaching assay. Values are means \pm standard deviation of three experiments.

Fig. 2. Antioxidant activity of ME measured as percent scavenging of DPPH radical. Each value represents the mean \pm standard deviation of three experiments.

higher (389 µg/ml) IC₅₀ value has been reported [\(Wangens](#page-7-0)[teen, Samuelsen, & Malterud, 2004](#page-7-0)). A direct correlation between the total phenolic content and DPPH radical-scavenging $(r^2 = 0.98, p < 0.05)$ was found in mint extract. Other researchers have also reported positive correlation between free radical-scavenging activity and total phenolic compounds [\(Wangensteen et al., 2004; Zheng & Wang,](#page-7-0) [2001](#page-7-0)). Phenolic compounds are effective hydrogen donors, which make them good antioxidants. Besides, they possess ideal structural properties for free radical-scavenging activities.

3.2.3. Hydroxyl radical-scavenging activity

Among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to the adjacent biomolecules ([Gutteridge, 1984](#page-6-0)). Mint extract was effective at quenching the hydroxyl radicals generated in both the site and non-site specific assays (Fig. 3). There was a linear correlation ($r^2 = 0.93$, $p < 0.05$) between concentration of ME and OH -scavenging activity within the applied concentrations. The 50% inhibition was accomplished with 498.3 μ g/ml of mint extract in the non-site specific assay. Flavonoids are very effective scavengers of hydroxyl radicals [\(Bravo, 1998\)](#page-6-0) and a good correlation between the total flavonoid content and hydroxyl radicalscavenging $(r^2 = 0.90, p < 0.05)$ was found in ME. The effectiveness of ME in inhibiting deoxyribose degradation due to hydroxyl radical damage was greater in the nonsite-specific assay than in the site-specific assay. Free radicals are related to various physiological and pathological events, such as inflammation, immunization, aging, mutagenicity and carcinogenicity ([Namiki, 1990](#page-7-0)). It has been suggested that compounds, which possess antioxidant activity, in addition to reducing rancidity in the food, when consumed can inhibit mutation and cancer because they

Fig. 3. Hydroxyl-scavenging activity of ME. Values are means \pm standard deviation of three experiments.

can scavenge a free radical or induce antioxidant enzymes ([Hochstein & Atallah, 1988](#page-6-0)).

3.2.4. Superoxide radical-scavenging activity

Numerous biological reactions generate superoxide rad-Numerous ordegien reactions generate superoxide radical (O_2^{\bullet}) which is a highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potential precursors of highly reactive species, such as hydroxyl radical, and thus the study of the scavenging of this radical is important. Superoxide radicals were generated in a PMS-NADH system and assayed by the reduction of NBT. Fig. 4 illustrates the superoxide radical-scavenging ability of ME. Mint extract showed a concentration-dependent ($r^2 = 0.71$, $p < 0.05$) scavenging of superoxide radicals and had an IC_{50} value of 48.8 µg/ml.

Fig. 4. Superoxide anion-scavenging activity of ME. Values are $means \pm standard deviation of three replicate experiments.$

Extracts from other plant sources, such as tea [\(Yagi et al.,](#page-7-0) [1999\)](#page-7-0), potato peel ([Singh & Rajini, 2004\)](#page-7-0) and garlic ([Wei &](#page-7-0) [Lau, 1998](#page-7-0)), have also been reported to have good superoxide radical-scavenging activity.

3.3. Iron-chelating activity

During lipid peroxidation, iron generally acts as a catalyst and thus the ability of ME to bind the metal was tested by assessing its ability to compete with ferrozine for ferrous ion and avoiding the formation of the coloured complex with absorbance peak at 562 nm. Though ME had a high phenolic acid content, indicating the presence of OH groups capable of chelating metal ions, it showed low iron-chelating activity (Fig. 5). This is in accordance with [Dorman et al. \(2003\)](#page-6-0) who found that, compared with other Mentha species, Mentha spicata L. var. crispa had a lower iron-chelation capacity. These results suggested that the antioxidant mechanism of mint extract was not due to iron chelation and is possibly due to chain termination by radical-scavenging activity. The protective effect of antioxidants in biological systems are ascribed mainly to their capacity to scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes and inhibit oxidases [\(Kulk](#page-7-0)[arni, Aradhya, & Divakar, 2004; Pratt, 1992](#page-7-0)).

3.4. Reducing power

Different studies have indicated that the antioxidant effect is related to the development of reductones, which are terminators of free radical chain reactions [\(Dorman](#page-6-0) [et al., 2003; Singh & Rajini, 2004](#page-6-0)). The reducing activity of ME is shown in Fig. 6 and it can be seen that concentration of ME had a significant ($r^2 = 0.99$, $p < 0.05$) effect. At a concentration of 500 μ g/ml, ME had a reducing activity which was half that of ascorbic acid at the same concentration. A positive correlation ($r^2 = 0.965$, $p < 0.05$) was

Fig. 5. Iron-chelating activity of ME. Values are means \pm standard deviation of three replicate experiments.

Fig. 6. Reducing power of ME. Values are means \pm standard deviation of three replicate experiments.

observed between the reducing power and the antioxidant activity of the mint extract by the β -carotene bleaching assay system. Our results are in accordance with other investigators ([Tanaka, Kuie, Nagashima, & Taguchi,](#page-7-0) [1988\)](#page-7-0) who have also reported that antioxidant properties are concomitant with the development of reducing power.

3.5. Effect of processing methods on ME

The chief objective of this study was to investigate the potential of ME as a natural antioxidant in radiation-processed meat. When used as an antioxidant, ME is added to meat prior to irradiation. Hence the effect of irradiation at doses normally employed for meat irradiation was checked on ME. The antioxidant potential of ME depends on its phenolic content and the radical-scavenging capacity. There was no significant ($p > 0.05$) difference between irradiated (at the doses studied) and non-irradiated ME on the total phenolic content and DPPH-scavenging activity [\(Table 1\)](#page-6-0). Our results are in agreement with those obtained by [Murcia et al. \(2004\),](#page-7-0) who found that irradiated spices did not show significant differences ($p > 0.05$) in antioxidant activity with respect to non-irradiated samples. In traditional Indian preparations, mint leaves are added to lamb meat for flavour either in raw chopped form or as ground paste with other spices. It is then either pressure-cooked or heated at temperatures of $100\,^{\circ}\text{C}$. Thus, the effect of autoclaving on ME was investigated. The total phenolics and radical-scavenging activity of ME was retained $(p > 0.05)$, even after autoclaving, indicating that the active constituents were resistant to thermal denaturation and hence could be used as an antioxidant in meat processes involving heating. In the case of spices, such as garlic, ginger, cloves, basil and pepper, it has also been reported that heating at 100 $\mathrm{^{\circ}C}$ or above did not result in any loss of antioxidant activity ([Shobana & Naidu, 2000; Tomaino et al.,](#page-7-0) [2005\)](#page-7-0).

Table 1 Effect of irradiation and autoclaving on ME^a

Process	Total phenolics	$%$ DPPH scavenging ^b
<i>Irradiation</i>		
0 kGy	21.1 ± 0.30	72.2 ± 0.50
2.5 kGy	20.3 ± 0.31	$71.1 + 0.52$
5 kGy	20.6 ± 0.30	71.1 ± 0.51
10 kGy	20.9 ± 0.32	71.1 ± 0.51
Autoclave	20.3 ± 0.30	$71.2 + 0.51$

^a 1% ME was irradiated/autoclaved. Data are expressed as mean \pm standard deviation of three experiments.

 b Concentration of ME used was 50 µg/ml.</sup>

3.6. Lipid peroxidation in radiation-processed lamb meat

The level of malonaldehyde, a product of lipid peroxidation, is often measured in order to assess the extent of rancidity that has occurred in biological systems. Fig. 7 shows the TBARS values of radiation-processed lamb meat treated with mint extract during chilled storage. Non-irradiated control samples had significantly lower ($p \le 0.05$) initial (0) day) TBARS than irradiated samples. These results concur with other researchers who have also reported an increase in lipid peroxidation of radiation-processed meat and meat products (Ahn, Jo, Du, Olson, & Nam, 2000; Lambert, Smith, Dodds, & Charbonneau, 1992). In order to ascertain the antioxidant potential of ME in radiation-processed meat, it was added to the meat before irradiation. Two different concentrations of ME were taken to determine the lowest most effective dose that was comparable with the maximum permissible (0.02%) concentration of BHT. Immediately after irradiation meat containing 0.05% and 0.1% ME had TBARS 18% and 34% less, respectively, than untreated irradiated meat. On chilled storage, there was significant increase in TBARS in irradiated samples not containing ME/BHT. Antioxidant treatment significantly $(p < 0.05)$ reduced lipid oxidation for all irradiated samples throughout the storage period. After 4 weeks of chilled

Fig. 7. TBARS of ME-treated irradiated lamb meat during chilled storage. Values are means \pm standard deviation of three experiments.

storage, TBARS in irradiated meat containing ME (0.1%) or BHT (0.02%) was half of that in untreated irradiated meat. The antioxidant activity of ME (0.1%) was not significantly ($p > 0.05$) different from that of BHT (0.02%) in irradiated (2.5 kGv) lamb meat. There are only some reports on the use of ME as an antioxidant. [Mari](#page-7-0)[nova and Yanishlieva \(1997\)](#page-7-0) reported that Mentha spicata L. was able to retard the autooxidation of sunflower oil at 100° C. Decrease in enzymatic lipid peroxidation has also been reported by aqueous plus alcoholic extract of mint ([Shobana & Naidu, 2000\)](#page-7-0).

The antioxidant activity of natural compounds is now intensively studied due to the current growing demand from the pharmaceutical and food industries, which are interested in natural bioactive compounds that possess health benefits. Plant sources may bring new natural products into the food industry with safer and better antioxidants that provide good protection against oxidative damage, which occurs both in the body and in the processed food. Addition of ME to irradiated lamb meat may be able to prevent lipid peroxidation, in addition to imparting flavour and taste to the meat. This combination treatment could have an important economic impact as it might reduce losses attributed to spoilage and also allow the products to reach new and distant markets.

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