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Antioxidant potential of curry (*Murraya koenigii* L.) and mint (*Mentha spicata*) leaf extracts and their effect on colour and oxidative stability of raw ground pork meat during refrigeration storage

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ABSTRACT

The aim of this study was to investigate the antioxidant activity of different solvent extracts of curry and mint leaf and their effect on colour and oxidative stability of raw ground pork meat stored at 4 ± 1 °C. The results indicated that among the two individual leaf categories, the ethanol extract of curry leaf (EHEC) and the water extract of mint leaf (WEM) showed higher DPPH and $ABTS^{+}$ activity. EHEC also exhibited the highest total phenolic contents while these were the lowest for WEM. WEM showed the highest superoxide anionic scavenging activity (%). The pork meat samples treated with EHEC and WEM showed a decrease in the Hunter *L*- and *a*-values and *a* increase in *b*-value during storage at 4 °C. However, the pH and TBARS values were higher in control samples irrespective of storage periods. In conclusion, EHEC and WEM have the potential to be used as natural antioxidants to minimise lipid oxidation of pork products.

1. Introduction

Lipid oxidation is one of the major factors affecting the quality of precooked meat products. Lipid oxidation is influenced by the composition of phospholipids, the amount of polyunsaturated fatty acids, the presence of metal ions, oxygen, haem pigments, mechanical processes, and the addition of salt during processing. Lipid oxidation is initiated when polyunsaturated fatty acids react with molecular oxygen via free radical chain mechanism forming peroxides (Devatkal, Narsaiah, & Borah, 2010). Myoglobin oxidation causes discolouration, which influences consumer acceptance in the market place. However, the fatty acid compositions of phospholipid fractions of the muscle cells are especially important in determining the stability of meat, because oxidative changes are initiated from the membrane components of muscle cells (Ahn, Ajuyah, Wolfe, & Sim, 1993). Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have successfully been used to prevent lipid oxidation in meat but recent report on health claims of these synthetic chemicals has necessitated research on effective alternatives particularly from natural sources.

The use of natural preservatives to increase the shelf-life of meat products is a promising technology since many herbs, plants, fruits, and vegetables extracts or powders have antioxidant and antimicrobial properties. Antioxidant effects of oregano essential oil (Viuda-Martos, Ruiz-Navajas, Fernandez-Lopez, & Perez-Alvarez, 2010), grape (Sayago-Ayerdi, Brenes, & Goni, 2009), citrus fruit by-products (Devatkal & Naveena, 2010) and curry leaf powder (Biswas, Kondaiah, & Anjaneyulu, 2006) have been investigated for their use in meat products. The antioxidant effects of grape seed, oregano extract, and rosemary in frozen vacuum packaged beef and pork was evaluated by Rojas and Brewer (2008). More recently Devatkal and Naveena (2010) evaluated the antioxidant effect of kinnow and pomegranate fruit by-product powders on the lipid stability and colour of model raw ground goat meat.

Curry leaf (*Murraya koenigii* L.) is native from east-Asian countries and mostly used as a flavour ingredient in a variety of products. The extracts contain monoterpene derived hydrocarbons and alcohols which have recently been recognised for their efficacy in providing significant antioxidant activity to human foods (Ningappa, Dinesha, & Srinivas, 2008; Rao, Ramalakshmi, Borse, & Raghavan, 2007). Mint (*Mentha spicata*) is an herb extensively used in Indian cuisine and also for curing several common ailments (Choudhury, Kumar, & Garg, 2006). Mint extracts were found to have very good antioxidant activity, which were comparable to that of the synthetic antioxidant, BHT (Kanatt, Chander, & Sharma, 2008). However, the antioxidant activity of curry and mint leaf

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extracts may vary depending on the extraction methods, purity, types and quantity of active compounds present according to climate, soil composition, plant organ, age, and stage in the vegetative cycle. As plant/herbs are rich in phyto-chemicals (polyphenols, vitamins, carotenoids, etc.), many food processors are incorporating them into the development of functional based processed products. So, the antioxidant activity of both curry and mint leaves needs to be assessed with suitable extractants, which are non-toxic to human.

Presently, the use of natural antioxidants in meat systems is limited. This is due to lack of availability, extra production cost, inadequate knowledge about their molecular composition and the amount of active ingredients in them, and unavailability of relevant toxicity data (Shahidi, Janita, & Wanasundara, 1994). In this study, the curry and mint leaf extracts were prepared with various solvents or solvent mixtures of different polarities to optimise the best extractant that lead to maximum production of a natural antioxidant. The antioxidant activity of the extracts of curry and mint leaf was assessed with a special emphasis for their effect on pork products.

2. Materials and methods

2.1. Materials

Fresh pork meat (composite samples) was obtained from the departmental slaughterhouse following scientific slaughtering techniques. The dressed carcases (65–70 kg) were chilled at 4 ± 1 °C for overnight, deboned manually, and then divided into small cubes ($5 \times 5 \times 5$ cm^3). For each of three replications, fresh meat samples were obtained separately. Fresh curry and mint leaves were obtained from the local supermarket. Standard gallic acid (SRL Chemicals, India), nitro blue tetrazolium (NBT), phenazin methosulphate (PMS), NADH (reduced nicotinamide adenine dinucleotide) (s.d. Fine Chemicals, India) Folin–Ciocalteau's reagent (CDH Chemicals, New Delhi, India), 2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid ($ABTS^+$), 1,1-diphenyl-2-picrylhydrazil (DPPH) and 2-thiobarbituric acid (Sigma–Aldrich, USA) used in the study were of analytical grade.

2.2. Preparation of mint and curry leaf powder and extracts

Fresh curry and mint leaves were obtained from the local supermarket, cleaned and washed thoroughly under running tap water. The excess water was drained out and the leaves were dried in a cabinet dryer (Macro Scientific Works, India) at 50 ± 2 °C for 8 h. Dried leaves were ground in a spice grinder (Inalsa make, India) to get fine particles of powder.

A total of six extracts, namely ethanol extract of curry leaf (EHEC), ethanol extract of mint leaf (EHEM), hot water extract of curry leaf (WEC), hot water extract of mint leaf (WEM), ethyl alcohol: hot water extract of curry leaf (1:1) (EHWEC) and ethyl alcohol: hot water extract of mint leaf (1:1) (EHWEM) were prepared for antioxidant activity (AOA) study. For preparation of EHEC, 0.5 g of the curry leaf powder was accurately weighed in a polypropylene centrifuge tube and then 10 ml of ethanol was added to it, and the tube was held for 10 min at room temperature (27 ± 1 °C), vortexed at high speed for 10 min, and finally centrifuged (Elico, USA) at 5000 rpm for 10 min. Supernatant was collected into a glass test tube, and the compounds were re-extracted with 10 ml of the same solvent followed by centrifugation once again as above. Both of the supernatants were pooled together and then passed through Whatman filter paper No. 42 (s.d. Fine Chemicals, India). The extract was stored at −20 °C for further studies. Other extracts of curry and mint leaf were also prepared in similar manner as mentioned for EHEC. All extracts were analysed for 2-2-azinobis-3-ethylbenthiazoline-6-sulphonic acid ($ABTS^+$) radical cations, 1,1 diphenyl-2-picrylhydrazil (DPPH) radicals, superoxide anion (SA) scavenging activity and total phenolics. The efficacy of curry and mint leaf extracts was determined based on the weight of respective dry powders.

2.3. Preparation of pork meat samples

About 2.0 kg of pork meat was minced twice (first minced through a 6 mm grinding plate followed by 4 mm plate) in a meat mincer (Kalsi motors, Ludhiana, India). After mincing, meat samples were divided into four different batches of 500 g each. The first and second batches were designated as control (meat without any extract) and sodium nitrite treated (contained 100 ppm of sodium nitrite; T_1) samples, while the other two treatments contained EHEC (T_2) and WEM (T_3) and were selected from each individual leaf category of all extracts following analysis of the total phenolic contents, DPPH, $ABTS^+$ and SA scavenging activity. A 5 ml of extract was added in each of the T_2 and T_3 samples, while the control and T_1 samples contained 5 ml distilled water to make up the volumes of the extracts used for the treatment groups. Sodium chloride (2%; w/w) was added in all samples. All batches of minced meat samples were mixed separately in an Inalsa food blender for 2 min. After completing the mixing, each sample was subdivided into five groups and then aerobically packaged in low density polyethylene bags. The samples were stored at 4 ± 1 °C and drawn at 2 days interval (0, 3rd, 6th, 9th, and 12th day) for evaluation of 2-thiobarbituric acid reacting substances (TBARS) values, pH and Hunter colour values.

2.4. Analysis of samples

2.4.1. DPPH radical scavenging activity

The DPPH radical scavenging activity of curry and mint leaf extracts was estimated by the method of Kato, Terao, Shimamoto, and Hirata (1988). DPPH can make stable free radicals in aqueous or ethanol solution. However, fresh DPPH solution was prepared in ethanol before every measurement. About 3.9 ml of DPPH (250 μM) solution was taken in a test tube, diluted with 1 ml of 0.1 M Tris–HCl buffer (pH 7.2) and then mixed well with 100 μl of curry /mint leaf extracts. The absorbance in time $t = 0$ min (t_0) was measured at 517 nm. The sample tubes were also incubated at room temperature (27 ± 1 °C) under dark for measuring the absorbance in time $t = 20$ min (t_{20}). Ethanol was used as blank sample. The free radical scavenging activity was calculated as a decrease in absorbance from the equation: Scavenging activity (%) = $100 - (At_{20}/At_0) \times 100$. Gallic acid (50–250 μM/ml) was used as a standard antioxidant.

2.4.2. SA scavenging activity

The superoxide radical scavenging activity was based on the reduction of nitro blue tetrazolium (NBT) in the presence of NADH and phenazin methosulphate (PMS) under aerobic condition (Kumar & Chattopadhyay, 2007). The reduction mixture contained 150 μl NBT (100 μM), 450 μl NADH (100 μM) and a sample extract 250 μg (200 μl). Total volume was made up to 1 ml with distilled water and then 1.9 ml of Tris–HCl buffer (0.02 M, pH 8.0) was added. The reaction was started by adding 100 μl of PMS (100 μM) and then the change in absorbance (A) was recorded at 560 nm after 1 min. Percent inhibition was calculated against a blank without the extract. SA scavenging activity (%) = $[(A_{Blank} - A_{Sample})/A_{Blank}] \times 100$. Gallic acid (30–90 μM/ml) was used as a standard antioxidant.

2.4.3. $ABTS^+$ radical scavenging activity

The free radical scavenging activity of sample extracts was determined by the ABTS radical cation decolourisation assay (Re et al., 1999). ABTS was dissolved in water to a 7 mM concentration. The ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and the mixture was allowed to stand in the dark at room temperature for 16 h before use. Because ABTS and potassium persulphate react stoichiometrically at a ratio of 1:0.5 (mol/mol), this results in complete oxidation of ABTS. Oxidation of ABTS commenced immediately, but the absorbance was not maximum and stable until 6 h had elapsed. The radical was stable in this form more than 2 days, when stored in the dark at room temperature. Prior to use, the stock solution was diluted with ethanol to an absorbance of 0.70 at t_0 ($t = 0$ min) and equilibrated at 30 °C exactly 6 min after the initial mixing. About 4.9 ml of ABTS working standard solution was mixed with 100 μl of plant extract/standard and the absorbance was measured after 20 min (t_{20}) at 734 nm. The $ABTS^+$ activity was calculated by using the formula: $ABTS^+$ activity (%) = $[(At_0–At_{20})/At_0] \times 100$. Gallic acid (200–500 μM/ml) was used as a standard antioxidant.

2.4.4. Total phenolics

The polyphenol content of curry and mint leaf extracts was quantified by Folin–Ciocalteau's reagent assay and expressed as gallic acid equivalents (μg GAE/g) (Yuan, Bone, & Carrington, 2005). Briefly, 100 μl of extract (250 μM concentration) were mixed with 2 ml of 2.0% Na_2CO_3 buffer and incubated at room temperature for two min. The total volume was made to 2.4 ml by adding distilled water. After addition of 100 μl of 1 N Folin–Ciocalteau's reagents the reactions tube was further incubated for 30 min at room temperature, and the absorbance was read at 720 nm. The amount of total phenolics was determined by a standard calibration curve ($y = 0.001x - 0.009$ and $r^2 = 0.992$; where, y = absorbance, x = gallic acid concentration, and r^2 = correlation coefficient) constructed using standard gallic acid solutions from 250 to 5000 μg/g concentrations.

2.4.5. TBARS value

The evaluation of lipid stability ($n = 6$) was performed by measuring TBARS at interval of 2 days during storage following the method of Witte, Krause, and Bailey (1970) with suitable modifications. Briefly, 10 g of sample were triturated with 25 ml of precooled 20% trichloroacetic acid (TCA) in 2 M orthophosphoric acid solution for 2 min. The content was then quantitatively transferred into a beaker by rinsing with 25 ml of chilled distilled water. They were well mixed and filtered through Whatman No. 1 filter paper (s.d. Fine Chemicals, Mumbai, India). Three millilitres of TCA extract (filtrate) were mixed with 3 ml of TBA reagent (0.005 M) in test tubes and placed in a dark room (27 °C) for 16 h. A blank sample was made by mixing 3 ml of 10% TCA and 3 ml of 0.005 M TBA reagent. The absorbance was measured at a fixed wavelength of 532 nm with a scanning range of 532 nm to 533 nm using a UV–vis spectrophotometer (Elico make, USA). The TBA value was calculated as mg malonaldehyde per kg of sample by multiplying the absorbance value with a factor of 5.2.

2.4.6. pH

The pH of meat sample ($n = 6$) was measured with a digital (Elico make, Model: Li 127) pH meter equipped with combined glass electrode (Trout et al., 1992) using 10 g of sample, homogenised with 50 ml distilled water, using pestle and mortar. The pH values were determined at 2-day intervals in the refrigerated samples. All determinations were performed in duplicate.

2.4.7. Instrumental colour evaluation

The colour profile was measured using Hunter Colour Lab (Mini XE, Portable type, USA) having setting of cool white light (D_{65}) and 2° was used to know Hunter *L*-, *a*- and *b*-values. Hunter *L* (brightness = 100/lightness = 0), *a*(+redness/−greenness), *b*(+yellowness/−blueness) values were recorded on the meat emulsion of all samples. The instrument was calibrated using black glass and white tile, provided with the instrument. The meat samples were kept inside the Petri dishes (85 mm × 12 mm) in duplicate, and then instrument was directly put on the surface of the meat emulsion at three different points for each Petri plates. The mean ($n = 18$) and standard error for each parameter were estimated.

2.4.8. Statistical analysis

Data were interpreted by analysis of variance (ANOVA) using 'SPSS-12.0' software packages as per the standard methods of Snedecor and Cochran (1994). Means of DPPH, SA, $ABTS^+$ and total phenolic contents were analysed using one-way ANOVA. Storage data of pH, TBARS and Hunter colour values were analysed using two-way ANOVA with Duncan's Multiple Range Tests. Three replicate experiments were carried out and the statistical significance was expressed at $P < 0.05$.

3. Results and discussion

3.1. Preparation of curry leaf and mint extracts and their antioxidant potentials

In preparation of sample extracts, the type of solvent is the most important factors to obtain a high quality natural antioxidant. The ideal solvent for extraction should be non-toxic, environmentally friendly, increase the extraction yield and have the ability to extract the most active components without unwanted loss. Considering these requirements, ethanol extracts, hot water extracts and combination of ethanol: hot water (1:1) extracts of curry and mint leaves were prepared and evaluated for their antioxidant activity (AOA). The AOA (Figs. 1–4) revealed that amongst the all extracts, the ethanol extract of curry leaf (EHEC) showed

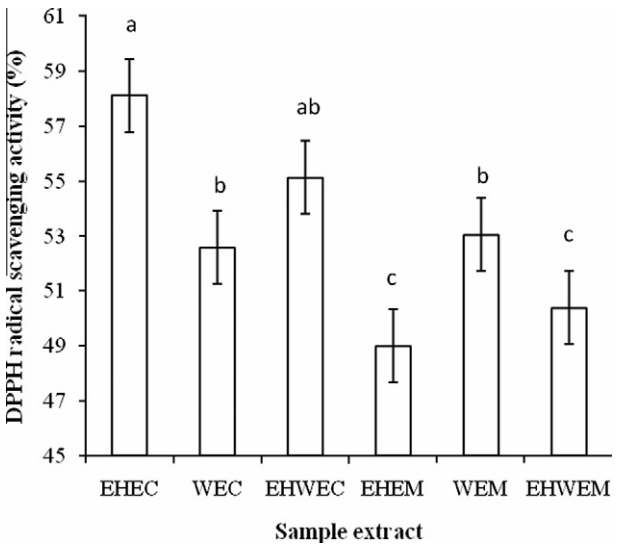

Fig. 1. DPPH radical scavenging activity (%) of curry and mint leaf extracts. EHEC, ethanol extract of curry leaf; WEC, water extract of curry leaf; EHWEC, ethanol: water (1:1) extract of curry leaf; EHEM, ethanol extract of mint leaf; WEM, water extract of mint leaf and EHWEM, ethanol: water (1:1) extract of mint leaf. $^{a–c}$ Mean ± SE with different small letter superscripts are different significantly at $P < 0.05$.

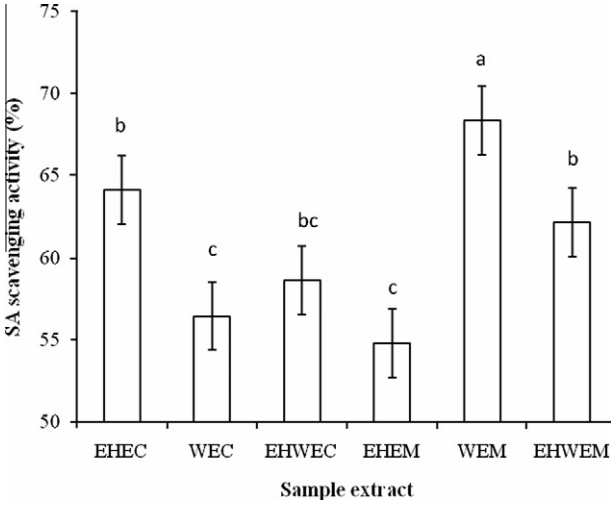

Fig. 2. SA scavenging activity (%) of curry and mint leaf extracts. EHEC, ethanol extract of curry leaf; WEC, water extract of curry leaf; EHWEC, ethanol: water (1:1) extract of curry leaf; EHEM, ethanol extract of mint leaf; WEM, water extract of mint leaf and EHWEM, ethanol: water (1:1) extract of mint leaf. [a–c]Mean ± SE with different small letter superscripts are different significantly at $P < 0.05$.

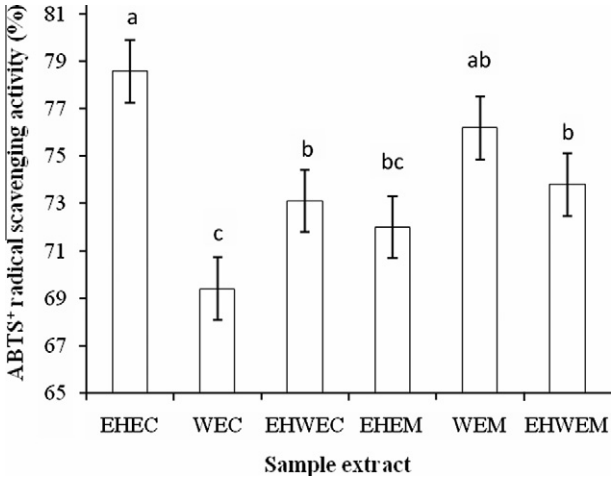

Fig. 3. $ABTS^+$ radical scavenging activity (%) of curry and mint leaf extracts. EHEC, ethanol extract of curry leaf; WEC, water extract of curry leaf; EHWEC, ethanol: water (1:1) extract of curry leaf; EHEM, ethanol extract of mint leaf; WEM, water extract of mint leaf and EHWEM, ethanol: water (1:1) extract of mint leaf. [a–c]Mean ± SE with different small letter superscripts are different significantly at $P < 0.05$.

highest AOA in DPPH assay as compared to other extracts. Overall, AOA increased in the following order: EHEM < EHWEM < WEC < WEM < EHWEC < EHEC. The DPPH scavenging activities were 49.01%, 50.40%, 52.59%, 53.06%, 55.14%, and 58.12% in EHEM, EHWEM, WEC, WEM, EHWEC and EHEC, respectively. When comparing among the different extracts of the curry leaf category, the EHEC exhibited greater DPPH, $ABTS^+$ and SA scavenging activities than the other two extracts. The EHEC was also shown to have a higher amount of total phenolics. So, it can be inferred that ethanol could have effectively extracted the major polyphenols (flavonols) present in curry leaf, which could be responsible for its AOA (Singh et al., 2011). Among the different extracts of mint leaf, WEM though had the lowest share in total phenolics, showing the highest DPPH, $ABTS^+$ and SA scavenging activity. This indicated that hot water is sufficient for the extraction of the major biologically active compounds responsible for the AOA of mint extract. In other words, the AOA of mint is not solely dependent on the total

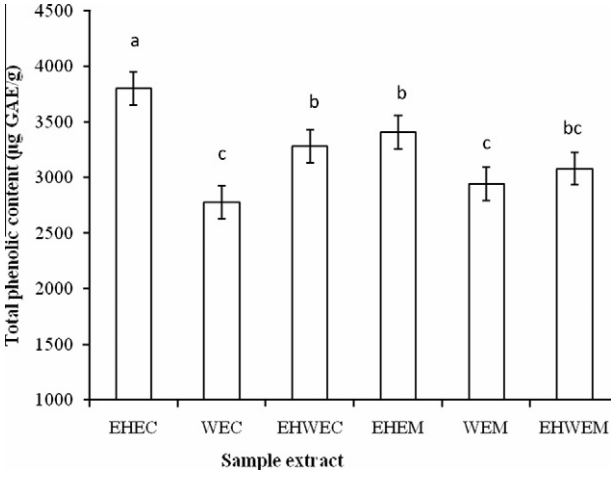

Fig. 4. Total phenolic contents (µg GAE/g) of curry and mint leaf extracts. EHEC, ethanol extract of curry leaf; WEC, water extract of curry leaf; EHWEC, ethanol: water (1:1) extract of curry leaf; EHEM, ethanol extract of mint leaf; WEM, water extract of mint leaf and EHWEM, ethanol: water (1:1) extract of mint leaf. [a–c]Mean ± SE with different small letter superscripts are different significantly at $P < 0.05$.

polyphenols though it was highest in EHEM. The radical scavenging activity of curry and mint leaf extracts have been reported in earlier studies (Kumar & Chattopadhyay, 2007; Ningappa et al., 2008). Kumar and Chattopadhyay (2007) reported a higher amount of total phenolic contents in water extract of mint leaf. Contradictory findings were also reported by Wang, Provan, and Helliwell (2004), as according to them, the water extract of mint leaf contained about 20% lower rosmarinic acid (phenolic compound) than the extracts with other solvents. Rao et al. (2007) also reported the radical scavenging activity and the total phenolics of curry leaf extract.

From this study it was found that EHEC and WEM exhibited greater AOA for each of the two individual leaf categories. So, both these extracts were used in preparing pork meat emulsions for colour and oxidative stability studies.

3.2. Effect of curry and mint leaf extracts on instrumental colour values

Changes in the instrumental colour values of raw ground pork meat during storage were depicted in Table 1. At day 0 of storage, the control exhibited highest colour values for all coordinates. The *L*-value (lightness) decreased significantly ($P < 0.05$) in the control as compared to other samples during storage. In the T_1 sample, the lightness, after an initial increase up to day 3, significantly ($P < 0.5$) decreased on day 6, indicating dark discolouration. Significant ($P < 0.05$) difference was also observed in lightness for T_2 and T_3 treatments. T_1 showed comparatively higher *a*-value but did not differed significantly from the other treatments. The *a*-value was found to increase in the control and the T_1 treatment on day 6 and 9, respectively, while the *b*-value deceased linearly and significantly with increasing storage time. The increase of the *a*-value indicated that the changes in colour from red to brown could be due to the formation of metmyoglobin as reported for only salt containing treatment (control). It has been further reported that salt greatly accelerates the process of meat discoloyration due to the pro-oxidative activity, which attributed its ability to release iron from haem pigments and other haem binding molecules. The curry and mint extracts treated samples also exhibited better colour stability as compared to those obtained for the well known preservative and colouring additive sodium nitrite. Devatkal et al. (2010) reported a reduction in the *L*-value and an increase in the *a*-values due to addition of natural antioxidants in chicken patties.

Table 1
Effect of EHEC and WEM on Hunter colour values of pork meat emulsion during refrigeration storage (4 ± 1 °C).

Parameter	Storage period (days)				
Treatment	0	3	6	9	12
L-value (lightness)					
Control	45.12 ± 1.22^{aA}	46.01 ± 0.92^{aA}	42.56 ± 1.17^{bA}	41.18 ± 0.86^{bA}	40.15 ± 0.47^{cA}
T_1	42.58 ± 0.71^{abB}	43.84 ± 0.66^{aB}	41.11 ± 1.13^{bAB}	40.31 ± 1.02^{bcAB}	38.77 ± 0.81^{cB}
T_2	38.28 ± 0.64^{aC}	38.35 ± 0.88^{abC}	38.78 ± 1.18^{bcB}	37.41 ± 0.44^{cC}	38.65 ± 0.62^{cdB}
T_3	36.42 ± 1.02^{aD}	36.69 ± 0.77^{aC}	37.57 ± 0.55^{aB}	35.88 ± 0.68^{aC}	35.49 ± 0.39^{aB}
a-Value (redness)					
Control	12.14 ± 0.23^{aA}	12.38 ± 0.19^{aA}	13.61 ± 0.41^{bA}	13.36 ± 0.30^{bA}	13.81 ± 0.33^{bA}
T_1	9.42 ± 0.31^{abB}	8.68 ± 0.24^{aB}	9.08 ± 0.32^{aB}	9.78 ± 0.23^{bB}	11.10 ± 0.29^{bAB}
T_2	8.37 ± 0.26^{aC}	8.08 ± 0.41^{aC}	8.58 ± 0.27^{aB}	8.14 ± 0.14^{aC}	8.01 ± 0.25^{aB}
T_3	7.89 ± 0.18^{aC}	8.04 ± 0.20^{aC}	7.96 ± 0.16^{aC}	8.10 ± 0.13^{aC}	8.18 ± 0.22^{aB}
b-Value (yellowness)					
Control	20.86 ± 0.44^{aA}	19.66 ± 0.64^{bA}	19.08 ± 0.77^{cA}	18.98 ± 0.46^{cA}	18.64 ± 0.37^{cA}
T_1	19.92 ± 0.51^{aAB}	18.80 ± 0.58^{bAB}	18.64 ± 0.52^{bA}	18.91 ± 0.38^{bA}	17.97 ± 0.42^{cA}
T_2	18.76 ± 0.39^{aB}	18.52 ± 0.41^{aB}	17.94 ± 0.61^{bAB}	17.46 ± 0.42^{bB}	17.46 ± 0.29^{bA}
T_3	16.28 ± 0.27^{aC}	15.78 ± 0.34^{aC}	15.48 ± 0.42^{abB}	15.36 ± 0.36^{bC}	15.52 ± 0.42^{abB}

$n = 18$; control = added salt only; T_1 = salt + sodium nitrite; T_2 = salt + ethanol extract of curry leaf (EHEC); and T_3 = salt + water extract of mint leaf (WEM).
$^{a-c}$Mean ± SE with different small letter superscripts on the same row are significantly different ($P < 0.05$).
$^{A-D}$Mean ± SE with different capital letter superscripts on the same column are significantly different ($P < 0.05$).

Table 2
Effect of EHEC and WEM on pH and TBARS values of pork meat emulsion during refrigeration storage (4 ± 1 °C).

Parameter	Storage period (days)				
Treatment	0	3	6	9	12
pH					
Control	6.03 ± 0.03^{c}	6.08 ± 0.01^{bcA}	6.20 ± 0.01^{bA}	6.37 ± 0.01^{abA}	6.52 ± 0.02^{aA}
T_1	6.01 ± 0.04^{c}	6.03 ± 0.01^{cB}	6.16 ± 0.02^{bAB}	6.23 ± 0.02^{abB}	6.25 ± 0.01^{aB}
T_2	6.05 ± 0.03^{c}	6.07 ± 0.04^{cA}	6.17 ± 0.01^{bA}	6.24 ± 0.01^{abB}	6.29 ± 0.04^{aAB}
T_3	6.04 ± 0.02^{c}	6.01 ± 0.02^{cB}	6.13 ± 0.01^{bB}	6.22 ± 0.02^{abB}	6.35 ± 0.01^{aAB}
TBARS values (mg malonaldehyde/kg)					
Control	0.548 ± 0.03^{eA}	0.763 ± 0.01^{dA}	0.980 ± 0.01^{cA}	1.26 ± 0.02^{bD}	1.417 ± 0.01^{aA}
T_1	0.424 ± 0.01^{eD}	0.541 ± 0.01^{dD}	0.742 ± 0.02^{cC}	0.938 ± 0.02^{bB}	1.167 ± 0.01^{aB}
T_2	0.449 ± 0.01^{dC}	0.482 ± 0.01^{cC}	0.725 ± 0.01^{bC}	0.844 ± 0.02^{aA}	0.961 ± 0.03^{aC}
T_3	0.487 ± 0.02^{eB}	0.662 ± 0.02^{dB}	0.780 ± 0.02^{cB}	1.062 ± 0.01^{bC}	1.32 ± 0.02^{aA}

$n = 6$; control = added salt only; T_1 = salt + sodium nitrite; T_2 = salt + ethanol extract of curry leaf (EHEC); and T_3 = salt + water extract of mint leaf (WEM).
$^{a-c}$Mean ± SE with different small letter superscripts on the same row are significantly different ($P < 0.05$).
$^{A-D}$Mean ± SE with different capital letter superscripts on the same column are significantly different ($P < 0.05$).

Rojas and Brewer (2008) have also reported a decrease in *b*-values in beef patties containing natural antioxidants. The same authors observed that *L*-value increased initially and remained constant later on.

3.3. Effect of curry and mint leaf extracts on pH

The results of effect of curry and mint leaf extracts on pH are presented in Table 2. At the beginning of storage, the pH was not significantly ($P > 0.05$) different between control and the treatments. However, on the day 3, the pH was significantly ($P < 0.05$) higher in the control sample. This could be attributed to the greater numbers of aerobic bacterial multiplication in the control than their counter part treatments, although no microbiological study was carried out.

3.4. Effect of curry and mint leaf extracts on TBARS

The effects of curry and mint leaf extracts on the TBARS values during refrigerated storage of pork meat are showed in Table 2. At day 0 of the shelf-life study, the TBARS values were significantly ($P < 0.05$) higher in the control sample followed by WEM (T_3), EHEC (T_2) and sodium nitrite (T_1) treatments. Significant ($P < 0.05$) increases in the TBARS values during the storage period were observed in the control and T_3. But in T_2, a significant difference was observed only between day 0 and day 6 of storage. In contrast, the control showed a significant increase in the TBARS values between all storage intervals. In the T_1 treatment, slight increases in the TBARS values were observed during the storage period. Treatment with curry leaf extract (T_2) showed lower ($P < 0.05$) TBARS during any day of storage than the control, T_1 and T_3 treated samples. The control samples showed a significantly ($P < 0.05$) higher TBARS values as compared to all other samples. These results indicated a strong pro-oxidant effect of salt and an anti-oxidative effect of sodium nitrite, curry leaf and mint leaf extracts in pork meat. Many studies have shown that the addition of salt to meat and meat products results in an increase in the TBARS values. Torress, Pearson, Gray, and Shimokonki (1988) demonstrated that the salt was able to promote lipid oxidation in ground beef. Rhee and Zipin (2001) reported a pro-oxidant effect of salt during storage of beef and chicken. Possible reasons for salt promoted lipid oxidation are the reduction in the activity of antioxidant enzymes like catalase and glutathione peroxidases, the stimulation of lipid oxidation via iron activation by chloride ions, the displacement of iron molecule from the myoglobin structure by sodium ions thereby providing free iron for the catalysis of lipid oxidation (O'Neill, Albersheim, & Darvill, 1990).

The antioxidant properties of phenolic compounds were very well documented. A significant relation between phenolic content and antioxidant effect of curry leaf extract has been reported by

Ningappa et al. (2008). Similarly, Kanatt et al. (2008) observed a strong antioxidant effect of mint leaf extract. They also reported that the antioxidant potential of this extract was comparable to that of the synthetic antioxidant, BHT. Recently Devatkal et al. (2010) found a positive correlation between the phenolic content of plant by-products extracts and the reduction of TBARS in cooked goat meat patties.

4. Conclusion

Consumer's interest in meat products formulated with natural antioxidants has motivated researchers to evaluate the effectiveness of naturally occurring compounds in plant leaves and herbs for functional purposes. Curry and mint leaf extracts have a substantial amount of phenolic compounds and significant DPPH, $ABTS^{+}$ and superoxide anionic scavenging activity. This study clearly demonstrated the pro-oxidant effect of salt and antioxidant effects of sodium nitrite, the curry leaf and mint leaf extracts. Both these extracts significantly reduced auto-oxidation, and salt promoted lipid oxidation in raw ground pork meat during refrigerated storage. Among the two different extracts, the curry leaf extract was more effective in reducing manoladehyde formation. Therefore, it is concluded that curry leaf and mint leaf extracts could be successfully added to raw ground pork meat to function as natural antioxidants with added health benefits and increasing consumer appeal.

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