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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.