Comparative antioxidant effect of aqueous extracts of curry leaves, fenugreek leaves and butylated hydroxytoluene in raw chicken patties

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Abstract Antioxidant properties and use of aqueous extracts of curry leaves (*Murraya koenigii*) and fenugreek leaves (*Trigonella foenum-graecum*) as source of natural antioxidant in raw chicken meat were evaluated. Four treatments viz., I. Control (meat +2% salt), II. BHT (meat +2% salt +0.1% BHT), III. CLE (meat +2% salt +2% curry leaves extract) and IV. FLE (meat +2% salt +2% fenugreek leaves extract) were compared for lipid oxidation during eight days refrigerated storage. The average phenolic content was 59.2 and 52.8 mg/g gallic acid equivalent in CLE and FLE respectively. Free radical scavenging activity was 61.4 and 64.2% in CLE and FLE, respectively. CLE had significantly ($P<0.05$) higher reducing activity (2.4) as compared to FLE (2.2). Observation on lipid oxidation showed a significant ($p<0.05$) higher TBARS values in control patties than other patties containing BHT, CLE and FLE. Overall percent increase during storage period was highest in control followed by BHT, CLE and FLE. Both synthetic antioxidants and natural extracts significantly ($p<0.05$) decreased the TBARS values. The average percent decrease in TBARS values during storage was 18, 25.5 and 27.5 in BHT, CLE and FLE treatments, respectively. Therefore, it was concluded that water extracts obtained from curry leaves and fenugreek leaves could be explored as natural antioxidants in poultry meat and meat products.

Keywords Curry leaves · Fenugreek · Poultry meat · Oxidation · Natural antioxidants · TBARS values

Introduction

Convenience meat products are generally prepared from ground and pre-blended meat. Grinding and addition of salt are common processes used in the preparation of ground meat products which are generally marketed at refrigerated temperature (2–5°C). Grinding exposes lipid membranes to metal oxidation catalysts and salt has been demonstrated to accelerate lipid oxidation in chicken meat (O'Neill et al. 1999; Rhee and Ziprin 2001). Lipid oxidation is one of the major problems in the development of new convenient meat products (Gray and Pearson 1987). Lipid oxidation is often responsible for quality loss via formation of rancid flavour. Therefore, synthetic antioxidants like BHT and BHA have been used to prevent the oxidation problems in meat products. However, natural sources of antioxidants are considered to be safer than synthetic antioxidants. (Brewer 2011) Hence there is a growing interest in natural antioxidants in meat products. In recent years, various plant materials like fruit peel, leaves and extracts containing phenolic compounds have been demonstrated to be effective antioxidants in model systems. (Mansour and Ali 2000; Farag et al. 2006; Devatkal et al. 2011). Further, antioxidant effects of grape seed extracts, tea catechins, rosemary and sage and extracts of pomegranate and kinnow peels in poultry meat have been reported. (Brannan 2008; Devatkal et al. 2011).

Curry leaf (*Murraya koenigii*) is a traditional spice used in Indian cuisines. It is native to India and distributed in most of southern Asia. Phytochemical studies on the leaves stem bark, and root of this plant have shown the presence of large concentration of alkaloids, phenolic compounds and very high radical scavenging activity (Sharif et al. 2007; Tachibana et al. 2001). The antioxidant activity of curry leaf is attributed to mahanimbine, murrayanol and mahanine

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(Tachibana et al. 2001; Ningappa et al. 2008). Antioxidant effect of curry leaf powder in chicken and goat meat products has also been reported (Biswas et al. 2006; Das et al. 2011).

Fenugreek (*Trigonella foenum-graecum*) also known as methi in India is originally from southeastern Europe and western Asia, but today it is grown in many parts of the world (Altuntas et al. 2005). Fenugreek is known to contain alkaloids, flavonoids, salicylate and nicotinic acid, and polyphenols (Mansour and Ali 2000; Dixit et al. 2005; Naidu et al. 2011). These studies have also reported that fenugreek leaves contain high amount of total phenolics and tocopherols. Hettiarachchy et al. (1996) reported that beef samples with fenugreek extracts had better oxidative stability and fenugreek may be a promising natural antioxidant source in meat products.

Hence, an investigation was carried out to evaluate the antioxidant properties and antioxidant effect of water extracts from powders of curry leaves (CLE) and fenugreek (FLE) leaves in ground chicken patties stored aerobically for 8 days at 4±1°C.

Materials and methods

Meat and fruit by-products Fresh chicken meat (broilers of 7–8 weeks) was obtained from a local retail meat processing plant. Meat samples were chilled at 4 °C for 24 h before use. Fresh curry leaves (*Murraya koenigii*) and fenugreek leaves (*Trigonella foenum-graecum*) were also obtained from institute garden. Standard gallic acid (SD Fine Chemicals, Mumbai, India), thiobarbituric acid (MP Biomedicals Pvt. Ltd. Mumbai, India), and 1, 1, 3, 3- tetraethoxypropane, DPPH, phosphate buffer (Sigma Aldrich, New Delhi, India) were used in this study.

Preparation of extracts Curry leaves and fenugreek leaves were washed thoroughly under running water and cut into small pieces using a knife. About 10 g of leaves was mixed

with 25 ml boiled distilled water and left for 1 h in a water bath (60°C). The extract was filtered using a clean sterile muslin cloth and used for biochemical analysis and in further experiments.

Preparation meat patties About 3 kg of chicken meat was minced twice (10 mm plate followed by 8 mm plates using a meat mince (Sirman, Italy). After mincing, the meat samples (500 g each) were assigned to one of the following four treatments : I) Control (meat with salt and without antioxidant); II). BHT (meat with 2% w/w salt +0.1% BHT); III) CLE (meat with 2% w/w salt and 2% v/w curry leaves extract); IV) FLE (meat with 2% w/w salt and 2% v/w fenugreek leaves extract). Immediately after adding all ingredients, meat samples were thoroughly mixed and made into patties manually (75 g each). These patties were packed individually in low density polyethylene bags and stored at 4±1 °C for 8 days.

Estimation of total phenolics For total phenolics estimation, suitable aliquots (2 ml) 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 folin ciocalteu (1 N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance recorded at 725 nm after 40 min. The amount of total phenolics was calculated as gallic acid equivalent from the calibration curve using standard gallic acid solution (0.1 mg/ml) (Escarpa and Gonzalez 2001)

Estimation of free radical scavenging activity The ability to scavenge 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) radical by curry leaves and fenugreek extracts was estimated by the method Negi and Jayaprakasha (2003). Extract (100 µg) diluted with 0.1 M Tris–HCl buffer (pH 7.4) was mixed with 1 ml of DPPH (250 µM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and then absorbance was measured at 517 nm using a UV–VIS spectrophotometer (Model: Spectroscan 80 DV Biotech Eng. Management Company Ltd. U.K.). The scavenging activity was calculated by the following equation:

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

Estimation of reducing power The reducing power of the extracts was determined by the methods of Negi and Jayaprakasha (2003). A known concentration of extracts was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferric cyanide in a 10 ml test tube. The mixtures were incubated for 20 min at 50 °C. At the end of the incubation 2.5 ml of 10% trichloroacetic acid was added to the

mixture and centrifuged at 5000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 9.5 ml ferric chloride (0.1%) and absorbance was measured at 700 nm. Increase in absorbance indicated the reducing power of the extracts.

Thiobarbituric acid reacting substances (TBARS) value Lipid oxidization was monitored by measuring thiobarbituric acid

Table 1 Antioxidant properties of aqueous extracts of curry leaves and fenugreek leaves

Properties	Curry leaves extract (CLE)	Fenugreek leaves extract (FLE)
Total phenolics (mg/g GAE)	59.2±0.90 ^b	52.2±0.85 ^a
DPPH (free radical scavenging activity %)	61.4±1.3 ^a	64.2±0.78 ^b
Reducing activity	2.4±0.10 ^b	2.2±0.20 ^a

GAE Gallic acid equivalent; DPPH 2, 2-diphenyl-1-picrylhydrazyl. Means within a row with different superscript differ significantly ($p<0.05$); $n=10$.

reactive substances at an interval of 2 days during storage. TBARS were determined using extraction method described by Witte et al. (1970). TBARS were extracted in chilled 20% trichloroacetic acid. Thiobarbituric acid extracts of each sample were used for measuring the absorbance at 520 nm. 1, 1, 3, 3, tetraethoxypropane was used as standard for TBARS assay. TBARS numbers were calculated as mg of malonaldehyde per kg of meat sample. The percent reduction in TBARS by different treatments in comparison to control was also calculated arithmetically.

Statistical analysis Ten samples were evaluated for total phenolics, DPPH activity and reducing power. Data presented is the average of ten samples. The experiment on meat was replicated thrice and all parameters were measured in duplicate ($n=6$). All data were analyzed using statistical software, Agristat (Indian Agriculture Statistical Research institute, New Delhi, India). Storage data TBARS values were analyzed using two-way ANOVA with treatment and storage time as main effects. Statistical significance was identified at the 95% confidence level ($P<0.05$).

Results and discussion

Table 1 shows the antioxidant properties of curry leaves and fenugreek extracts. Curry leaves had a significantly ($P<0.05$) higher phenolic content. However, DPPH scavenging activity was significantly higher in fenugreek extract. The reducing power was significantly ($P<0.05$) higher in curry

leaves than fenugreek. Palaniswamy (2001) reported that water-ethanol extract of curry leaves comprised of a high polyphenolic content and the antioxidant activity may be the result of the synergistic action of all the components, rather than of a single entity of the extract. Nevertheless, the exact nature and the quantity of each of the constituents are yet to be characterized. Ningappa et al. (2008) reported a value of 54 mg/g of total phenolic content and 41% for the DPPH activity in water extract of curry leaves. Lee et al. (2002) reported that extracts of plants may well act as electron donors and they can react with free radicals to convert them into more stable products and terminate radical chain reactions. Also, it has been shown that the antioxidant effect exponentially increased as a function of the development of the reducing power (Tanaka et al. 1988). Similar studies have reported that natural antioxidants are involved in the termination of free radical reactions and exhibit reducing capacity (Wang et al. 2003). The reducing capacity might be due to their hydrogen donating ability (Shimada et al. 1992). This antioxidant activity is attributed to mahanimbine, murrayanol and mahanine from *M. koenigii* (Tachibana et al. 2001; Ningappa et al. 2008). Earlier report showed that the curry leaves had total phenolic content of 168 mg/g in alcohol-water extract (Tachibana et al. 2001). In their study they found that alcohol extract and the water extract had maximum reductive potential as measured by ferric ion reduction and the minimum was observed in the hexane extract.

Premanath et al. (2011) evaluated the antioxidant properties of fenugreek extract and reported a value of 1.5 mg/g catechin equivalent in aqueous extract. They have

Table 2 Overall changes in TBARS values (mg malonaldehyde/kg meat) of different raw chicken patties during storage period (Mean ± S.E)

Storage period (days)					
Treatments	0	2	4	6	8
Control	0.18±0.02 ^{a1}	0.70±0.06 ^{b3}	0.90±0.05 ^{c3}	1.25±0.12 ^{d2}	1.49±0.13 ^{d3}
BHT	0.13±0.01 ^{a1}	0.54±0.01 ^{b2}	0.67±0.09 ^{c2}	1.03±0.08 ^{d1}	1.30±0.11 ^{e2}
CLE	0.14±0.02 ^{a1}	0.51±0.07 ^{a2}	0.70±0.07 ^{a2}	0.90±0.06 ^{a1}	1.10±0.06 ^{a1}
FLE	0.15±0.01 ^{a1}	0.37±0.08 ^{b1}	0.58±0.06 ^{c1}	0.96±0.05 ^{d1}	1.08±0.05 ^{e1}

TBARS Thiobarbituric acid reacting substances. Means with different superscripts (numerical within a column and alphabets within a row) differ significantly ($p<0.05$); $n=6$. CLE Curry leaves extract. FLE Fenugreek leaves extract

also reported ferrous reducing and DPPH scavenging activity in fenugreek leaves extract. Dixit et al. (2005) reported significant antioxidant activity in germinated fenugreek seeds which may be due partly to the presence of flavonoids and polyphenols. Naidu et al. (2011) have reported a value of 85.8 mg/g of total phenolics in fenugreek extract and free radical scavenging activity of 50–70%. They observed that antioxidant activity appears to be directly correlated to the polyphenol content of fenugreek fractions. It is also known that the antioxidant activity of plants is due to phenolic and flavonoid compounds present in them (Tawaha et al. (2007).

TBARS values Mean TBARS values were significantly ($P<0.05$) higher in control than treated samples. Chicken patties with BHT were also having a higher TBARS values than CLE and FLE treated chicken patties. During all storage intervals TBARS was significantly higher in control followed by BHT and CLE showed the lowest increase in TBARS during storage (Table 2). After 8 days of storage, TBARS significantly increased in all samples but the levels of increase were significantly ($P<0.05$) lower in treatments containing BHT, CLE and FLE as compared to control treatment. Thus treatments with extracts and BHT showed a significant ($P<0.05$) lower TBARS values than control samples. Average percent increase in TBARS was maximum in control followed by BHT, CLE and FLE. Among treated samples, FLE showed the lowest increase in TBARS. However, CLE and FLE treated patties showed no significant difference in percent increase in TBARS during storage period. Similar observations on % reduction of TBARS revealed that BHT, CLE and FLE reduced TBARS by 18.8, 25.3 and 30%, respectively in comparison to control sample. Thus these extracts effectively delayed the formation of TBARS during refrigerated storage of chicken patties. These results clearly demonstrated that addition of CLE and FLE extract significantly reduced the oxidation in control sample and the antioxidant effect was higher than BHT. Similar to these findings, Das et al. (2011) reported that curry leaf powder (0.2%) significantly reduced lipid peroxides, free fatty acids (FFA) and thiobarbituric acid substances (TBARS) in raw ground and cooked goat meat patties during refrigerated storage. Biswas et al. (2006) have also observed the antioxidant effect of curry leaf powder in raw and cooked chicken meat. Hettiarachchy et al. (1996) reported in ground beef that fenugreek extracts delayed the induction period of oxidative rancidity and samples with fenugreek extracts had better oxidative stability and fenugreek may be a promising natural antioxidant source in meat products. Curry leaves are reported to contain carbazole alkaloids viz: murrayanine, mahanimbine, girinimbine, mukonine, murrayafoline-A which are reported to possess antioxidant activity (Ningappa et al.

2008). Further four more alkaloids are reported from curry leaves viz, euchrestine B, bismurrayafoline E, mahanine, mahanimbicine (Indu and Menon 2010). A significant antioxidant activity in fenugreek leaves might be due to the presence of flavonoids and polyphenols (Premanath et al. 2011). It has been reported that antioxidant properties of phenolic compounds is mainly due to the redox properties which play as an important role as free radical scavengers, reducing agents and complex of pro-oxidant metals (Butkhop and Samappito 2011). Wong et al. (2006) reported a linear correlation between antioxidant activity and total phenols in plant extracts. Similarly, Tawaha et al. (2007) found a positive correlation between total phenols and scavenging activity.

Conclusion

Curry leaf and fenugreek extracts showed significant level of total phenolics and antioxidant properties measured in terms of free radical scavenging and reducing activity. CLE and FLE significantly reduced the lipid oxidation in chicken meat during refrigerated storage. Further it was observed that antioxidant effect of these extracts was higher than that of BHT. Therefore it was concluded that extracts of curry leaves and fenugreek leaves could be successfully added to meat to function as antioxidant. Further research will be required to determine to know the molecules responsible the antioxidant properties in these extracts.

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