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Antioxidant effect of curry leaf (*Murraya koenigii*) powder on quality of ground and cooked goat meat

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Abstract: The antioxidant effect of curry leaf powder (CLP) was determined by assessing the formation of lipid peroxides, free fatty acids (FFA) and thiobarbituric acid substances (TBARS) in raw ground and cooked goat meat patties during refrigerated storage. pH, water holding capacity and cooking loss per cent were not affected by curry leaf powder when added in raw ground goat meat. Fresh goat meat with CLP had acceptable odour up to 5 days whereas in control sample it was up to 3 days. Raw goat meat with CLP had significantly lower free fatty acids content than control during 9 days refrigerated storage. CLP significantly inhibited the rate of lipid peroxides and TBARS formation in raw meat than control. CLP in cooked goat meat patties showed significant antioxidant effect as indicated by TBARS values measured by distillation as well as extraction method. CLP did not affect microbial population in raw and cooked goat meat during entire storage period. These results show that CLP at concentrations as low as 0.2% is a very effective inhibitor of primary and secondary oxidation products in raw ground and cooked goat meat patties and has potential as a natural antioxidant in raw and cooked meat systems.

Keywords: Curry leaf powder, antioxidant activity, free fatty acids, goat meat, quality

Introduction

Lipid oxidation is a major cause of muscle food deterioration as it decreases nutritional properties of foods since it involves the loss of essentially fatty acids and vitamins and the generation of potentially toxic reaction products such as the manoladehyde (MDA) and cholesterol oxidation products (COPs) (Morrissey *et al*., 1998; Tang *et al*., 2001). In addition, lipid oxidation affects essential sensory traits of meat product, causing flavour, colour and texture deterioration (Estevez *et al*., 2005). The development of so called warmed over flavour (WOF) in pre-cooked meat products was assumed due to high storage temperature and/or reheating before serving. Many substances have been investigated as potential antioxidant to prevent such lipid oxidation. However, the image of some synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and propyl, octyl, and dodecyl gallates has been worsened by the findings linking the use of these compounds to health risk (Tokusoglu and Basmacioglu, 2004; Das *et al*., 2006a). Development of various comminuted meat products offers a profitable utilization of tough meat and ground meat tends to become more rancid and brown more rapidly, due to pigment and lipid oxidation. Moreover, mechanisms for control of lipid oxidation has become increasingly important with the rise in popularity of pre-cooked and convenience foods (Das *et al*., 2006a). Consequently, substances derived from the plant kingdom such as dried herbs, grape seed extract and essential oils have been successfully used to reduce lipid oxidation in meat products (Estevez *et al*., 2005, Brannon, 2008). Many natural plant extracts contain primarily phenolic compounds, which are potent antioxidants.

Curry leaf (*Murraya koenijii*; Rutaceae) is a leafy spice characterizing authentic Asian-Indian cuisine and it is used in small quantities for its distinct aroma as well as for preservation purposes. Recently some studies have reported that carbazole alkaloids have several biological activities such as anticarcinogenic effects in dimethylhydrazine (DMH) treated rats (Farhath-Khanum *et al*., 2000), antiplatelet activity and vasorelaxing effects (Wu *et al*., 1998). Chevalier (1996) also reported curry leaf has medicinal value as traditionally used in eastern Asia. Interest in greater use of curry leaf has been stimulated since its high antioxidant potency was reported and this antioxidant activity is attributed due to mahanimbine, murrayanol and mahanine from *M .koenigii* (Tachibana *et al*., 2003; Ningappa *et al*., 2008). Chowdhury *et al*. (2001) reported that these alkaloids shown to have antimicrobial activity against gram positive and negative bacteria, and fungi. Use of curry leaf powder as antioxidant in ground and pre-cooked muscle foods on oxidative stability has been poorly reported. In this experiment, we used curry leaf powder instead of different extracts due to its major use in culinary purpose in the form of green

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leaf or dry powder. Therefore, this work was design to investigate the efficacy of CLP as antioxidant and antimicrobial in ground goat meat and precooked goat meat patties during refrigerated storage and to evaluate the TBARS values determined by two different methods (distillation and extraction).

Materials and Methods

Source of meat samples

The samples, comprising mostly *Longissimus dorsi*, *Semimembranosus* and *Semitendinosus* muscles of male (of about 12-16 months age) goat carcasses, slaughtered as per the approved guidelines of Animal Ethics Committee in the Institute slaughterhouse, were collected within 5 hrs of slaughter, packed in low density polyethylene (LDPE) bags. The samples were placed in a refrigerator at 4±1°C for about 22 hrs.

Preparation of control and treated meat samples

The meat pieces, after conditioning, were trimmed off separable fat and connective tissue, cut into small cubes and minced using automatic meat mincer by passing through 5 mm plates (Talleres Raman Model P-22, Barcelona) to obtain ground goat meat (GGM). The products were collected in sterile mixing bowls. Ground goat meat was divided into 2 groups. The curry leaf powder (CLP) was used as final percentage of total meat weight: 1) no CLP (control), 2) CLP at 0.2%. CLP was mixed uniformly with ground goat meat and meat was reground through 5 mm plates and packaged in LDPE bags, sealed and stored in a refrigerator 4±1°C up to 9 days. Two packages for each treatment were selected on sampling days 0, 3, 5, 7 and 9 to determine various physico-chemical and microbiological quality parameters.

Preparations of spice mix and curry leaf powder

Dried spices namely aniseed, black pepper, capsicum, caraway seed, cardamom, cinnamon, clove, coriander, cumin, ginger and turmeric were procured from local market and oven dried at 50°C for 2 hrs before grinding and mixing. Spice mix was stored in polyethyleneterephthalet (PET) bottle for experimental use. Fresh matured leaves of *M. koenigii* plant from Institute campus were collected and sun-dried after removal of extraneous matter. The leaves were kept in oven at 50°C for 2 hrs and then ground mechanically and sieved through a fine mesh (U.S.S. 30 # mesh screen). The curry leaf powder was stored in a bottle for further use.

Preparation of goat meat patties

The separated lean obtained from the carcasses was packed in high-density polyethylene bags and stored at -15±1°C till used for patties preparation. Before use, meat was thawed overnight at 4±1°C. Minced goat meat was transferred into a bowl chopper (Seydelmann, Model type K20, Germany) for preparation of meat emulsion in batches of 3 kg using standard recipe (Das *et al*., 2006b). After the preparation of emulsion, it was divided into 4 groups: 1). No text ingredients (control), 2) 1.5% spice mix only, 3) 0.2% CLP only and 4) mixture of spice mix and CLP. These emulsions were mixed separately in Hobart paddle type mixer (Model No.50, USA) for three min.

About 70 g of emulsion was moulded in a Petridish (75 mm diameter and 15 mm height) to form patties and was cooked in a pre-heated oven at 180±5°C for 15 minutes after which they were turned and allowed to get cooked for 10 more minutes till internal temperature reached about 75 ± 2°C recorded by a probe thermometer (Labware Scientific, Inc, USA). The patties were packed in low-density polyethylene pouches and stored at refrigerated temperature (4±1ºC). TBARS values of the product were evaluated using both distillation and extraction method at an interval of 5 days. The experiment was replicated thrice.

pH determination

pH of raw ground and cooked meat patties was determined by blending 10 g of sample with 50 ml of distilled water for 1 min using a tissue homogenizer (Model PT-MR-2100, Kinematica AG, Switzerland) at 8000 rpm for 1 min. The pH of the suspension was recorded by dipping combined glass electrode of digital pH meter (Systronics, µ pH system 361, Delhi, India).

Water holding capacity and cooking loss

Water holding capacity (WHC) of ground goat meat was estimated by a centrifugation method (Wardlaw *et al*., 1973). Cooking loss (CL) percent of the samples was estimated by heating approximately 25 g of ground meat sample individually placed inside polyethylene bags at 80°C for 20 min using a thermostatically controlled water bath. Samples were cooled for 15 min under running tap water. They were taken from the bags, dried with filter paper and weighed. Cooking loss was expressed as the percentage loss related to the initial weight (Das *et al*., 2006a).

Colour and odour score

Color score was determined by using a 5-point scale, where 1=pale pink, 2=pink, 3=pinkish red, 4=bright red and 5= reddish brown. Whereas sensory score for the meat odour was obtained using a 5-point scale, where 1=very unpleasant, 2=moderately unpleasant, 3=moderately pleasant, 4=pleasant and 5=very pleasant (Das *et al*., 2006a).

Lipid oxidation

Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS), lipid peroxides and free fatty acids. The distillation as well as extraction method described by Tarladgis *et al*. (1960) and Witte *et al*. (1970) respectively, were followed to measure 2-thiobarbituric acid-reacting substances (TBARS) values of the raw ground and cooked meat. The values were expressed as mg malonaldehyde/kg of sample. The methods described by Koniecko (1979) were followed for the determination of peroxide value and free fatty acids (FFA).

Microbiological examination

For microbiological examination, a representative of 10 g ground meat sample was withdrawn and homogenized (Model PT-MR-2100, Kinematica AG, Switzerland) aseptically using 90 ml 0.1% peptone water (APHA, 1984) and serial dilutions were made using 0.1% sterile peptone water. Total aerobic bacteria and psychrotrophs counts were enumerated on duplicate pour plates of Plate Count Agar (Hi-Media, Mumbai, India), which were incubated at 37°C for 48 h and 5°C±1 for 10 to 12 days respectively. After appropriate incubation, plates having 25 to 250 colony-forming units (CFU) were counted and multiplied by the dilution factor to determine CFU/g of meat.

Statistical analysis

Three trials were conducted for each experiment. The statistical design of the first experiment was 2 (treatment) × 3 (replication) × 5 (storage periods) combination with randomized block design and data were analyzed by analysis of variance (ANOVA) using Linear Model Procedure of SPSS software 10. Duncan's multiple range test (DMRT) and critical difference (CD) were determined at 5% and 1% significant level. The statistical design for the second experiment (cooked meat patties) was same, except four treatments were conducted.

Results and Discussion

Curry leaf powder on pH, Water holding capacity (WHC) and cooking loss

Effect of curry leaf powder on pH, water holding capacity and cooking loss of ground goat meat during refrigerated storage is presented in Table 1. Addition of curry leaf powder in ground goat meat (GGM) did not change the pH. Biswas *et al*. (2006) observed that pH was not affected due to curry leaf powder added in ground poultry meat. pH values of control and treated samples increased significantly with increasing storage period. The increase in pH during the storage period may be due to growth of Gram-negative bacteria such as *Pseudomonas*, *Moraxella*, *Acinetobacter*, etc (McDowell *et al*., 1986). Jay (1996) also reported increase in pH during storage and this could be due to accumulation of metabolites by bacterial action on protein and amino acids. The pH recorded in the present study were in range reported by Verma and Sahoo (2000) in ground chevon during refrigerated storage.

Water holding capacity (WHC) is known to be one of the major quality characteristics of fresh meat, as it affects some major characteristics of cooked meat such as potential drip loss, technological quality, appearance and sensory properties. WHC was not influenced by CLP. There was a significant linear decrease in WHC of the meat samples during storage period. Similar result was reported in ground buffalo meat during refrigerated storage (Das *et al*., 2006a). Cooking loss (CL) percentage of ground goat meat was not significantly different from the control sample. CL of goat meat increased linearly during storage period for 9 days. It increased from 43.92 on day 1 to 51.58 on day 9 of the storage period. This trend of CL may be due to a decrease in the solubility of protein during storage and post mortem enzymatic

Table 1. Effect of curry leaf powder on pH, water holding capacity and cooking loss of ground goat meat during storage

Treatments	Storage period (days)						Treatment effect
	1	3	5	7	9	SEM	
pH							NS
Control	5.70^{c}	5.84^{b}	5.88^{b}	6.05^{a}	6.09^{a}	0.03	
CLP	5.69^{d}	5.75^{cd}	5.92^{bc}	5.99^{ab}	6.07^{a}	0.03	
Water holding capacity (%)							NS
Control	15.43^{a}	12.87^{b}	10.99^{c}	5.66^{d}	3.13^{e}	0.85	
CLP	15.38^{a}	12.88^{b}	10.82^{c}	5.50^{d}	2.79^{e}	0.86	
Cooking loss (%)							NS
Control	43.37^{e}	45.76^{d}	48.09^{c}	50.13^{b}	51.67^{a}	0.56	
CLP	43.92^{e}	45.03^{d}	47.89^{c}	49.67^{b}	51.58^{a}	0.51	

Means bearing different superscripts in a row differ significantly (P<0.05),
NS= Non-significant, SEM- Standard Error of the Mean.

Table 2. Effect of curry leaf power on colour, odour, free fatty acids and peroxide values of ground goat meat during storage

Treatments	Storage period (days)						Treatment effect
	1	3	5	7	9	SEM	
Colour Score$^{\$}$							NS
Control	4.59^{a}	4.28^{b}	3.57^{c}	2.83^{d}	2.07^{e}	0.18	
CLP	4.62^{a}	4.38^{b}	3.34^{c}	3.22^{d}	2.41^{e}	0.16	
Odour Score$^{\#}$							NS
Control	4.48^{a}	3.72^{b}	2.99^{c}	2.42^{d}	1.82^{e}	0.19	
CLP	4.32^{a}	3.96^{b}	3.25^{c}	2.86^{d}	2.00^{e}	0.17	
Free fatty acids (%)							**
Control	0.37^{d}	0.58^{c}	0.65^{c}	0.78^{b}	0.93^{a}	0.04	
CLP	0.34^{e}	0.46^{d}	0.55^{c}	0.57^{bc}	0.71^{a}	0.03	
Peroxide values (meq/kg)							
Control	1.74^{a}	2.21^{b}	2.52^{c}	3.01^{d}	3.56^{e}	0.03	**
CLP	1.52^{a}	2.04^{b}	2.17^{b}	2.61^{c}	2.82^{d}	0.05	

Means bearing different superscripts in a row differ significantly (P<0.05),
**P<0.01; NS= Non-significant, SEM- Standard Error of the Mean
#Odour score based on 5 point scale, where 1=pale pink, 2=pink, 3=pinkish red, 4=bright red and 5= reddish brown.
$Colour score based on 5 point scale, where 1=very unpleasant, 2= moderately unpleasant, 3=moderately pleasant, 4= pleasant and 5=very pleasant.

hydrolysis of ATP (Hamm, 1970).

Curry leaf powder on colour and odour score

Colour and odour of fresh meat are also important criterion for its acceptability. There was no significant difference in colour and odour of control and CLP treated ground goat meat (Table 2). During refrigerated storage period, the visual colour declined significantly as the storage days progressed. The colour score of treated sample was 4.62 on day 1, which decreased to 2.41 whereas in control sample, it was 4.59 on day 1 and 2.06 on day 9. Das *et al*. (2006a) also reported similar results in ground buffalo meat in refrigerated storage. In the present study, the visual colour crossed the borderline (3.00) in control sample after day 5 while the sample containing CLP had colour appealing till the end of day 7. These findings were in agreement with the observation of Verma and Sahoo (2000) in tocopherol preblended ground chevon in chilling storage.

CLP though did not significantly influence odour score but improved odour score than control sample. As indicated in Table 2, the CLP treated sample was acceptable upto 5 days, odour score being 3.35. On the contrary, the control sample was below the acceptable level (3.00) on day 5 that means it was acceptable up to three days. The odour score started declining linearly as the storage period increased. Das *et al*. (2006a) reported similar trend of odour score in ground buffalo meat during refrigerated storage. In general, minced goat meat sample maintained the desirable dour up to 5 days and lost the odour sometime between day 5 and 7 (Verma and Sahoo, 2000).

Curry leaf powder on free fatty acids and peroxide values

Curry leaf powder treated sample had significantly lower free fatty acids content (0.31 to 0.71) as compared to control sample (0.37 to 0.93) (Table 2). It is clear that addition of 0.2% CLP to ground goat meat is sufficient to lower the free fatty acids content during refrigerated storage. Pearson (1968) reported that minced beef had FFA content in the range of 0.38 to1.74% and a maximum acceptability limit of 1.8% FFA in view of their progressive increase during storage. In the present study, FFA content of minced goat meat was well within this limit. Again Lea (1962) also reported that flavour deterioration in beef or mutton was noticeable when the acidity of extracted fat reached 2-3% as oleic acid. FFA content of meat sample significantly increased during refrigerated storage. Previous workers also reported the increasing trend of FFA content of buffalo meat (Rao and Kowale, 1988) and goat meat (Verma and Sahoo, 2000) during 9 days refrigerated storage. Recently, Das *et al*. (2008) also reported increase of FFA during refrigerated storage of goat meat patties.

The peroxide value generally serves as a useful indicator of the extent of oxidation of lipids, fats and oils. An advantage of peroxide value determination is that it directly measures the lipid peroxides, which are primary lipid oxidation products (Shantha and Decker, 2001). Similarly, addition of CLP significantly reduced the peroxide value of ground goat meat during storage. There was a significant linear increase in peroxide value of the meat samples during refrigerated storage. In this study, peroxide value in control sample increased from 1.74 ppm day 1 to 3.56 ppm on day 9 whereas incase of treated sample, it increased from 1.52 to 2.82 ppm (Table 2). Verma and Sahoo (2000) found a positive correlation between peroxide value and thiobarbituric acid reactive substances (TBARS) number of chevon during refrigerated storage.

Curry leaf powder on thiobarbituric acid values of raw ground goat meat

Figure 1 shows the effect of curry leaf powder on TBARS values measured by distillation and extraction method of raw goat meat during refrigerated storage. Addition of curry leaf powder, into minced goat meat at a concentration of 0.2% resulted in a significant ($P<0.05$) reduction of TBARS values compared to the control. There was a linear increase of TBA value in both control and treated goat meat during refrigerated storage. TBARS value increased from 0.65 on day 1 to 1.12 on day 9, whereas in control sample it was 0.74 to 1.43 during storage. Extraction method showed significantly lower mean TBARS value than distillation method. The control meat sample was not acceptable after day 3 while CLP treated sample could be acceptable up to day 7 as revealed by TBA value of 0.99. Control ground goat meat exhibited significantly higher rate of TBRAS formation than CLP containing raw goat meat during storage. The acceptability limit of TBARS value in this study was 1.0. Earlier workers reported that meat sample containing TBARS value less than 1 possesses no off odour (Tarladgis *et al*., 1960).

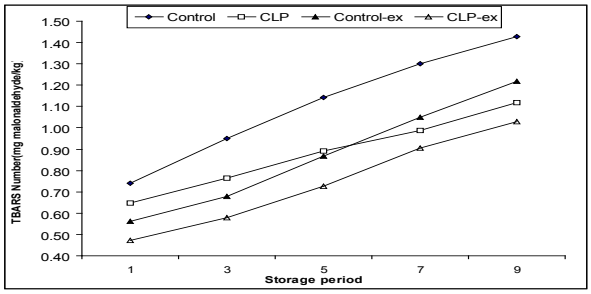

Figure 1. Effect of CLP on TBARS value (distillation and extraction method) of raw ground goat meat during refrigerated storage (ex-extraction method)

Spice mix (SM) and CLP on TBARS values of cooked goat meat patties

CLP in cooked goat meat patties showed significant antioxidant effect as indicated by TBARS values estimated by distillation and extraction method (Fig 2 and Fig 3). In distillation method (DM), the control sample had significantly higher lipid oxidation throughout the storage period than SM, CLP + SM and CLP treated patties. CLP along with SM revealed enhanced antioxidant effect than CLP alone in goat meat patties during 20 days refrigerated storage. Control patties like raw ground meat showed significantly higher rate of TBARS formation than patties containing CLP during storage. TBARS value increased from 0.36 on day 1 to 0.89 on day 20 whereas in control sample it was 0.49 to 1.11 mg malonaldehyde/kg sample during 20 days storage. Tachibana *et al*. (2003) reported that carbazole alkaloids of CLP have potent antioxidant activity. Enhanced antioxidant activity of CLP extract was due to higher phenolic content (Ningappa *et al*., 2008). Again Lee *et al*. (2002) noted that enrichment of phenolic compounds within the plant extract is correlated with their enhanced antioxidant activity.

In extraction method (EM), similar trend of antioxidant activity of CLP was observed in goat meat patties during 20 days storage period. Patties with CLP and SM had significantly lower TBARS value than others. In DM, significantly higher TBARS value had been observed in all samples during storage period than EM. Such difference in TBA value between these methods was due to estimation of malonaldehyde at different wavelength (Tarladgis *et al*., 1960). Biswas *et al*. (2006) also report that higher TBARS values in DM than in EM in cooked chicken patties during storage. Besides this, heating in DM increases aldehyde quantities from lipid precursors. Ulu (2004) reported that heating of samples during distillation promotes further oxidation leading to additional malonaldehyde and other TBA reacting substances. This study reported above clearly indicates that CLP is heat stable and withstands meat processing temperatures

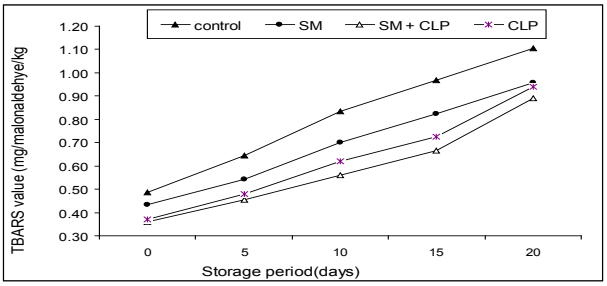

Figure 2. Effect of spice mix and CLP on TBARS values (distillation method) of cooked goat meat patties during refrigerated storage

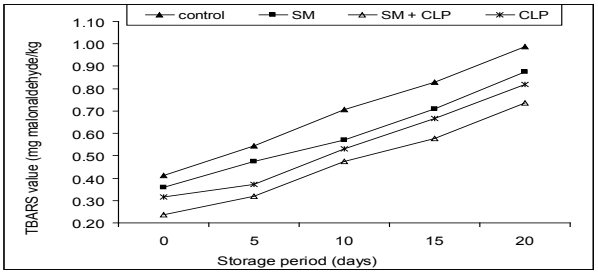

Figure 3. Effect of spice mix and CLP on TBARS values (extraction method) of cooked goat meat patties during storage

Curry leaf powder on microbial quality of raw and cooked goat meat

Curry leaf powder did not show any antimicrobial effect when mixed with ground goat meat during chilling storage. Microbial count increased significantly with increase in storage period in both control and treated sample. The total plate count ranged from 4.57 to 7.01 log10 cfu/g and 3.92 to

6.39 log10 cfu/g in case of psychrotrophs during 9 days storage period. Contrasting to this finding, Chowdhury *et al*. (2001) reported the antimicrobial activity of carbazoles from *M. koenigii*. Similarly, in case of cooked goat meat patties, there was no significant difference in microbial count between control and treated patties. Patties with dry spice mix had slightly lower microbial load but that was not significant. Total plate count and psychrotrophs count observed in this study were well with in the acceptable limit in cooked meat products. Biswas *et al*. (2006) did not find any antimicrobial effect of CLP both in fresh and cooked chicken meat.

Conclusion

Use of curry leaf powder inhibits the formation of free fatty acids, lipid peroxide and thiobarbituric acid reactive substances in ground goat meat that does not affect pH, water holding capacity and cooking loss during storage. Shelf life of the ground goat meat could be extended up to 5 days as compared to 3 days in control sample during refrigerated storage. This study also shows that CLP has effective antioxidant activity in cooked goat meat patties during storage. Curry leaf powder seems to have no anti-microbial effect on fresh as well as cooked goat meat during storage. However, further studies especially in regard to use of different CLP extracts as antioxidant and pro-oxidative effect of NaCl are needed in order to reinforce this conclusion.

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